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PREVALENCE OF THE YELLOW FEVER MOSQUITO, AEDES CALOPUS, IN THE SOUTHERN PART OF PERU

LAWRENCE H. DUNN

Ovid, New York

Received for publication December 4, 1922

The writer, while engaged as Entomologist and Supervising Inspector in the Recent Campaign Against Yellow Fever in Peru, had the opportunity of making a survey to determine the mosquito index at the principal seaports and towns in the coastal region of the southern half of Peru.

This work was carried out, by order of Dr. Henry Hanson, Director of the Campaign, for the purpose of ascertaining the distribution and prevalence of the yellow fever mosquito, *Aedes calopus* (*Stegomyia calopus*), at the various places mentioned. Efforts were also to be made to instruct the inhabitants of the towns visited in the measures necessary for the control of this species of mosquito and, if possible, to induce them to institute such measures.

As this survey and the findings therefrom may be of interest to those concerned in mosquito work, this article is therefore presented. The region in which this survey was made differs considerably in climatic and topographic conditions from other places where work has been carried on in connection with the yellow fever mosquito; consequently a brief description may not be amiss.

The coastal zone of the southern half of Peru consists of the region lying between the Pacific Ocean and the Andes Mountains and extending southward from Lima to the southern boundary of Peru. This territory is approximately 600 miles in length and in some sections up to 90 miles in width. The foothills, or knots from the Andes, extending from the Cordilleras toward the coast cause considerable variation in the width. This coastal zone is bare and abrupt near the sea and extends eastward as extensive flat plains, broken by valleys, between the chains of foothills. It may be classed as an arid region as rain never, or very rarely, falls there. It is therefore in many sections, where irrigation is not feasible, an uninhabited, treeless desert, devoid of practically all vegetation.

A number of rivers flow from the mountains down through the valleys and discharge into the Pacific. Some of these have their headwaters high enough in the Andes to be fed by the heavy rains and thawing snowfields of the higher regions and are therefore more or less permanent throughout the year. Although none of them is of large volume, they are of great importance as it is in the sections of valleys traversed by them that irrigation is practiced and agriculture carried on.

It is also mainly along the most important of these rivers that the towns and villages are located, each of the larger rivers having usually a village at its mouth, at the coast for a seaport, while further inland may be one or more larger and important towns with many smaller villages between. Railroads winding up the valleys connect the ports with the inland villages, but, owing to the chains of foothills extending at right angles to the coast and acting as barriers to land traffic from north to south, there are practically no railroads, and but few wagon or automobile roads, running parallel with the coast. This causes nearly all coastwise traveling to be dependent upon ocean transportation. Because of this topographical configuration, the populated areas are mostly confined to the valleys, with extensive desert plateaus and chains of foothills intervening.

This trip of inspection was made during January and February, 1922. Sanitary Inspector Guillermo Olivari, of Callao, accompanied and assisted the writer while engaged in this work.

The word mosquito as used in this article specifically denotes the yellow fever mosquito, *Aedes calopus*, and does not signify any other species.

The percentages of houses with breeding as given herewith were arrived at by dividing the number of houses inspected into the number in which mosquito breeding was encountered. Thus each house in which larvae or pupae were found was counted as one house with breeding, regardless of whether this breeding was occurring in one container or in six. This is somewhat different from the usual system of determining the prevalence of mosquito breeding in the majority of surveys made in connection with this species of mosquito, as in the most of such surveys made in the past the percentage of containers with breeding has usually been given. With such a system if five containers in a hundred examined were found to contain breeding it would be counted as 5 per cent of the containers with breeding, whereas all five of these containers may have been in the same house, there being but actually one house with breeding occurring. Therefore the *percentage of houses* with breeding in a town is quite probably of greater value in denoting not only the prevalence, but also the distribution, of mosquito breeding, than the *percentage of containers* with breeding would be.

Owing to the arid conditions and lack of rainfall in this region, no accidental containers, such as tin cans, broken bottles, etc., filled by rains, were encountered. All water found in the containers examined had been placed there purposely and for use. The greater number of these containers consisted of large earthenware vessels, *botijas* and *tinajas*, wooden barrels, kegs. etc., all of which ordinarily served as receptacles for fresh water used for domestic purposes.

Our inspections of wells, excepting those that were very shallow and therefore could be readily examined, were owing to lack of time, necessarily very cursorily carried out, usually consisting of lowering a pail on a rope and drawing it up full of water and an examination of the latter for mosquito breeding.

The Canete Valley, about 70 miles south of Lima, was the nearest point to Lima that inspections were made during this particular survey. The three towns, San Vicente, Imperial and San Luis, all situated a few miles inland in this valley, were inspected.

San Vicente. At this place 120 houses were visited and 155 containers examined, breeding being found in 55 houses, or

45.83 per cent of the number visited, and in 66 containers. Fourteen of the houses had no containers other than pails or kerosene tins which owing to small capacity were usually emptied and refilled several times each day, when in daily use, and were therefore seldom found to contain breeding. About 36 of the houses had a pipe borne water supply.

Imperial. Of 140 houses inspected in this town, 62.85 per cent were found to be positive for mosquito breeding. Of 170 containers examined, 98 were found to contain breeding. There was no pipe borne water supply in this town, all water being obtained from irrigation streams passing through it.

San Luis. One hundred houses were visited here and breeding encountered in 62. Of 146 containers examined, 92 were found positive. Eight of the houses had no containers other than pails. Irrigation streams passing through the town furnished the water supply.

Tambo de Mora. This is a small port town that serves the Chincha Valley, about 35 miles south of Canete. Nearly every house in this town was supplied with a well. The majority of these were very shallow, a number of them being but mere holes in the ground, about 2 feet in diameter and the same in depth. Practically all of them were located in close proximity to the houses, usually in the rear close to the kitchen. Fifty houses were inspected and breeding encountered at 23. Of 43 wells examined, 17 were positive. But 7 containers were found and all contained breeding.

Chincha Alta. This is a town of about 8000 inhabitants, located approximately 8 miles inland from the Port of Tambo de Mora. Of 203 houses visited, 68.42 per cent were positive for breeding, being present in 213 containers out of 264 examined. While two large irrigation streams passing through the town furnish part of the water used, a large percentage of the houses have a pipe borne water supply. This comes from three privately owned wells situated in different sections of the town. It is pumped from these wells into large storage tanks whence it passes through pipes to the sections of the town supplied. This supply is not constant, the water being turned on only during certain hours each day and shut off the remainder of the day while the storage tanks are being refilled and more water comes into the wells. As a consequence of this daily interruption of the water supply, each house having a water tap had a container to hold a reserve supply of water for use during the hours when the supply in the pipes was cut off. These containers were usually located beneath the taps and the majority of them, being apparently but seldom cleaned, seemed to be prolific mosquito breeding places. It was also found that breeding was occurring in the storage tanks and the larvae were undoubtedly being distributed, to some extent, through the pipes to the houses. Owing to these factors prevailing, the pipe borne water supply in this town seems to be more advantageous for mosquito breeding than otherwise.

Pisco. This port town in the Chunchanga Valley about 20 miles south of Tambo de Mora, is the principal port between Callao and Mollendo and has approximately 4000 inhabitants. Mosquito breeding was encountered in 41.05 per cent of the 302 houses inspected. Of 262 containers examined, 142 were found positive. The greater number of the houses in the town have wells to furnish their water supply. These are from 6 to 12 feet deep and contain from 1 to 5 feet of water. The majority of them were uncovered and from all indications there was but little doubt but that many of them are prolific breeding places for mosquitoes. Of the 214 of these wells examined, breeding was found in 10. Several of the wells that were covered emitted numbers of adult mosquitoes when the covers were removed. However, these latter were not recorded as positive breeding places as no larvae or pupae were found. Numerous adult mosquitoes were also much in evidence in this town, manifesting their presence in the hotel at which we sojourned by biting on all favorable occasions.

San Andres. A small village of about 80 houses, chiefly inhabited by fisher folk, located about 4 miles down the coast from Pisco. The water is obtained from wells and no regular containers were in use. Fifty houses, 34 wells and 1 container were inspected in this village but no mosquito breeding was found.

Ica. Located in the Ica Valley, about 60 miles south east of Pisco, this town has about 10,000 inhabitants. A river on one side of the town and a large irrigation stream on the other, furnish water for some of the houses, but the chief source of supply is the wells, of which nearly every house has one. A total of 414 houses were visited in this town and mosquito breeding found in 46.61 per cent of them. Four hundred and sixty containers were examined and breeding found in 256. A cursory examination was made of a number of the wells but no breeding was found, although many of the people claimed that they frequently found larvae in water taken from them.

Mollendo. This port is second to Callao in importance among Peruvian ports and has approximately 1000 houses. The water supply is piped from near Arequipa, a distance of nearly 100 miles, and is controlled by the railroad, "Ferrocarril del Sur de Peru," as it is brought to the city primarily for use by the railroads and the surplus sold to the city. It is piped to the majority of the houses and there are also a number of public taps located in the streets. As at Chincha Alta this supply is not constant but is cut off during part of each day; containers beneath the taps to hold the day's reserve supply were in evidence in a large percentage of the houses. Inspections were made at 350 houses and breeding found in 55.71 per cent of them. A total of 560 containers were examined and 252 found positive. The water was quite turbid which made the examinations very difficult and because of this, it is quite possible that in many instances we were unable to find breeding where it was actually present, which makes the percentage that was found probably somewhat lower than would have been found had the water been clear. Barrels were the most common containers in use at this place, many of them being very large, heavy and clumsy to overturn at each time when cleaned, which may account for some of them having the appearance of seldom being cleaned.

Cocochacra. This is a small village of about 100 houses, located in the Tambo Valley about 20 miles inland from Mollendo. The water supply consists of the Tambo Riber and several irrigation streams passing near the village. Although 50 houses were visited and 74 containers inspected no mosquito breeding was found.

Chucarapi Hacienda. This hacienda was located about 2 miles from Cocochacra. Visits to 36 houses and examinations of 47 containers failed to reveal any breeding.

Ilo. This town is about 60 miles south of Mollendo and is the most southerly port in Peru. It has approximately 225 houses. The water supply, which was a very turbid one at the time of our visit, is piped from the Moquegua River, about 2 miles distant from the town. But few of the houses are piped and the water is taken from taps located at various public places in the town. As at other places this supply is not constant at all hours of the day and many containers are necessary. Mosquito breeding was encountered in 28.66 per cent of the 150 houses visited, and of 161 containers examined, 51 proved positive.

Moquegua. Located 61 miles inland from Ilo at an altitude of about 4000 feet, this town has approximately 900 houses and nearly 4000 inhabitants. Water is piped from a stream in the hills down to public taps located at convenient points in various streets. Many containers were in use as but few of the houses are piped. Of 305 houses inspected, 71, or 23.27 per cent, were found to have mosquito breeding. Eighty-seven containers out of 504 examined contained breeding. A great many large, heavy and cumbersome *tinajas,* 4 feet and more in height and over 3 feet in diameter, of the kind used for storing wine or oil, were in use as water containers at this town.

During this survey a total of 2270 houses were visited and 331 wells and 2810 containers examined, these latter including 966 *botijas,* 812 *tinajas,* 782 barrels, 120 tanks, 89 filter stones and 41 tubs. In addition to these regular containers many other receptacles in temporary use as water containers were examined. Mosquito breeding was found in 994 houses, more than 43.78 per cent of the total number visited, occurring in 27 wells and 1264 containers. These containers consisted of 618 *botijas,* 189 *tinajas,* 359 barrels, 41 tanks, 31 filter stones, 13 tubs, 3 grindstone boxes, 2 pails, 2 iron pots, 2 flower vases and on the tops of 4 empty barrels, where a small amount of water had been placed to prevent shrinkage of the wood.

The maximum and minimum temperatures at each place were observed during our stay. The maximum of the trip was 90°F., recorded at Ilo and the minimum 48°F., at Moquegua.

The foregoing data applies only to the yellow fever mosquito, *Aedes calopus*, and does not include *Culex* and other varieties of mosquitoes found breeding during this survey.

Dr. Enrique Caballero, Medical Inspector for the Campaign, having been detailed by Dr. Hanson to make an inspection of the southern part of Peru for the purpose of determining the possible presence of yellow fever also accompanied us on this inspection trip and by his active coöperation in many ways was of great assistance to us for which I wish to express my thanks and appreciation.

SUMARIO

El autor estuvo empleado como entomólogo e inspector sanitario durante la reciente campaña contra la Fiebre Amarilla en el Perú y tuvo la oportunidad de hacer una investigación con el objeto de determinar el índice de mosquitos en los puertos y poblaciones de la región costanera de la mitad meridional del Perú. Incluye una descripción de las condiciones climatológicas y topográficas de los lugares visitados y resúme sus observaciones como sigue:

Fueron examinadas 2270 viviendas, 331 pozos y 2810 receptáculos. Estos últimos incluían 966 botijas, 812 tinajas, 782 barriles, 120 tanques, 89 piedras de filtrar y 41 cubas. Se observaron criaderos de mosquitos en 994 casas, o sea, en mas del 43.78 por ciento del total visitadas y también en 27 pezos y 1264 receptáculos diversos. Estos receptáculos consistían en 618 botijas, 189 tinajas, 359 barriles, 41 tanques, 31 piedras de filtrar, 13 cubas, 3 depósitos de piedras de amolar, 2 baldes, 2 calderos de hierro, 2 floreros y en la superficie de 4 barriles vacíos en donde una pequeña cantidad de agua se había echado para prevenir la contracción de la madera. La temperatura máxima observada fué de 90 grados F., en Ilo y la mínima de 48 grados F. en Moquegua.

Los datos que anteceden están relacionados con el mosquito de la Fiebre Amarilla y no incluyen el Culex u otras variedades que también encontramos en nuestro paso.

STEGOMYIA INDICES AND THEIR VALUE IN YELLOW FEVER CONTROL[1]

M. E. CONNOR AND W. M. MONROE

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The epidemiologist divides a country in which yellow fever is present into infected, infectible, and non-infectible areas. The infected area is further subdivided into endemic and epidemic zones. In the endemic zone the disease is constantly present; in the epidemic zone it may be rampant or there may be only an occasional case, and there will be evidence that the cases have been imported from a nearby seed-bed. This paper is limited to a consideration of certain methods of control in infected areas.

An epidemic zone does not always become endemic, because in many instances the factors necessary to establish endemicity are not constantly present or do not remain in the required relation one to the other for sufficiently long periods.

The continuance of yellow fever in endemic areas is due to the regular importation of non-immune persons to whom infected mosquitoes have access, rather than to the newborn who have heretofore been held responsible. The theory that endemicity is maintained primarily by the young, and more especially by the newborn, is not, in our experience, borne out by facts. That this element of the population is a factor in perpetuating the infection cannot be denied, but that it plays a major part, as has been generally accepted heretofore, is extremely doubtful. Many cities have automatically cleansed themselves of yellow fever in spite of a normal birth-rate, and in centers of endemicity an increase or decrease in the birth-rate has not been found to

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[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Board of the Rockefeller Foundation.

be paralleled by a corresponding fluctuation in the number of yellow fever cases reported.

Further, we have observed that cases of this disease increase in an area coincidently with the number of non-immunes entering it, and that as the flow of non-immunes decreases there is a falling off in the incidence of the disease. As will be shown later the active areas in an endemic center will be where both infected mosquitoes and non-immune persons congregate, namely, in the district of cheap lodging-houses and hotels, where water-containers are largest and most numerous.

Endemic centers of yellow fever are of two classes: the city endemic center and the farm or hacienda center. There is this difference between city and hacienda endemicity: the urban center remains distinctly circumscribed, a fact which has caused yellow fever to be classed as a place disease, whereas in hacienda territory the infection moves from one place to another and is limited by the distribution of the haciendas and the mosquito line. Both centers will continue active only so long as the component factors necessary for establishing endemicity continue in proper proportions, and this balance is influenced, in fact controlled, by the commercial activities in the area. Whenever trade is brisk we may look for a series of yellow-fever cases increasing in number until a "boom stage" is reached, accompanied by an epidemic of the disease. This epidemic will continue until an economic crisis intervenes, when the number of cases will diminish in proportion to the severity of the crisis. Here the chief factor in the reduction of the disease is not the control of the Stegomyia mosquito, but the coming of fewer non-immunes into the area and a consequent failure of the human host.

The conditions necessary to establish hacienda endemicity are: (1) A group of plantations whose crops are harvested at different periods and at such intervals as will insure the continuance of the causative organism of yellow fever. (2) Free labor coming from non-infected zones and allowed to pass without restriction from one hacienda to another.

The term free labor is used here in contradistinction to indentured labor. In countries where the latter form of labor is used, employees are not permitted to go at will from one area to another, and this restriction tends to check the spread of yellow fever. Obviously it is quite difficult if not impossible for the disease to become endemic in haciendas which have practically no communication with adjoining areas. Should the disease be introduced into such communities, it would automatically burn itself out.

It is rare that yellow fever is present in a hacienda for long periods even though free labor is employed, for new labor will avoid a place where the infection is known to exist. Persons remaining in the infected community will in time become immune and the disease will die out, nor will it reappear until a new generation has come into being. Therefore, it will be seen that hacienda endemicity is not so easily established as might be supposed on first thought, and this is in agreement with actual experience in that there are not many true endemic hacienda infections recorded.

COMPUTING STEGOMYIA INDICES IN CONTROL CAMPAIGNS

The present-day method of controlling yellow fever in a community where a modern water system cannot be installed is to effect a reduction in the number of *Aedes calopus* by destroying the larvae. This is accomplished mainly by placing larvae-consuming fish in the containers in which fresh water is stored. Other measures employed are the sealing of water-tanks, the oiling of such containers as cannot be otherwise treated, the separating of larvae from water by straining through cloths, and, where water is not too expensive, the emptying of larvae-infested containers.

To measure the progress of a campaign it is the practice to chart the number of *Aedes calopus* breeding-places found upon each inspection of a district. From the daily reports of such charting are deduced various indices. At the present time there are a number of Stegomyia indices in common use, all expressed in percentages. That most widely employed is the *house index*, more properly called the *density index,* which is the ratio of the total number of containers in which breeding is found to the number of houses visited. This gives the highest index of the three, for often two or more breeding-places are found in the same house. The lowest index, on the other hand, is reached by comparing the total number of containers examined with the total number of breeding-places found. This is called the *deposit index.* Between these two is the *index of infected houses,* in which the emphasis is placed on the number of homes which are found to harbor water containing larvae and pupae.

The method of computing indices is as follows: If on a given date 2812 houses are visited and of this number 177 are found to have mosquito-breeding receptacles, the infected house index will be 6.2. If on this same day the number of fresh-water receptacles examined is 7770, and of this number 258 are found breeding mosquito larvae, the deposit index will be 3.3. The density index will be the number of houses visited,—in this instance 2812—divided into 258 (the number of deposits found breeding mosquitoes), giving a percentage of 9.1.

Of these indices the most valuable would seem to be that of the containers, for it is the only one in which there is not a variable factor. Each container is considered a separate entity, and the percentage of those infested is a true percentage, whereas either of the other indices may be misleading because a house may have two or more deposits of water—we have known one house to have twenty-three—and all or none of these may contain larvae.

In times of an epidemic the index of infested houses may be of importance, for one breeding-place will supply adult mosquitoes quite as well as will half a dozen, although not in the same number. Should a person capable of infecting mosquitoes be domiciled in a house containing a breeding receptacle, the chances are that sooner or later that home will contain infected Stegomyiae.

Good workmen in every profession like to have exact standards by which to judge their work, and so those engaged in the various yellow-fever campaigns have attempted to fix a standard of measurement for their operations. It is generally conceded that with a container index of 10 per cent or less, a "safety zone" has been reached. This figure should be modified, however, with reference to the place for which such an index is given, as the propagation of yellow fever depends just as much on the percentage of non-immunes in an area as on the "critical" number of Stegomyiae present;[2] for without doubt a given number of Stegomyia mosquitoes would be more dangerous in a city in which there were only 5 per cent of immunes than they would be in a like city where 95 per cent of the inhabitants were immune to yellow fever. But no strict arithmetical ratio can be made from such figures, for it has been shown time and again in endemic centers where spot maps have been used to indicate yellow fever cases, that such cases are not scattered all over the city in proportion to the population, but are grouped in the locality of the hotels and the cheaper boarding-houses and rooming-houses, where immigrants and tourists congregate and where water-containers are large and numerous, since hotels need a greater water-supply than private dwellings. This means that infected mosquitoes are in practically every instance gathered where the non-immune population, thus tremendously increasing the chances that a non-immune will be selected for feeding. Thus it may happen that instead of a 95 to 5 chance against conveying disease, an infected mosquito may have as much as a 50 per cent chance, if such a percentage of non-immunes are domiciled in the lodging-houses. From this it may be seen that a ''safety index'' for an epidemic center need not be so much lower than for an endemic center as one would think.

In any city the suburbs and certain districts are populated by families who have lived in the community long enough to have saved sufficient money to buy land and build a house. This requires time—time enough for the members of such families to become immune to yellow fever if the city happens to be an endemic center. New arrivals do not go to such sections, but remain in the lodging-house district. In conducting a control campaign in an endemic center, therefore, certain large districts may be practically disregarded, while in the districts which harbor the non-immunes the work must be as intense as in an epidemic center, where the non-immunes are scattered evenly through the town and where the large water-supplies of the hotels are not found. Control work in an endemic center, then, differs from that in an epidemic zone not so much as regards the safety index as regards the extent of the territory to which that safety index should be applied.

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[2] H. R. Carter, The mechanism of the spontaneous elimination of yellow fever from endemic centers. Annals of Tropical Medicine and Parasitology, **13**, no. 4, March 15, 1920.

That 10 per cent of the water-containers in an area are breeding-places should never be a basis for the belief that 10 per cent of the usual number of adult mosquitoes are emerging. It would be fair to assume that a much lower percentage are coming into existence. What the exact ratio may be can hardly be estimated, but its determination ought to be influenced by the following factors:

When there is free breeding in the water-containers of a city with no attempt at control, it often happens that the container index approaches close to 100, especially if there is a scarcity of water. It will also happen that in many of these containers the water will be actually saturated with larvae and pupae, that is, it will contain all the larvae and pupae that it is capable of supporting. This condition very rarely obtains where there is periodic inspection of water-containers at intervals shorter than the aquatic cycle of the mosquito's development. Instead, one is apt to find barrels and tins with only a few larvae, very rarely with pupae, and almost never with a pupal case. When this condition is compared with that of the uncontrolled breeding-places where myriads of adults are continually emerging, the importance of considering systematic control work when estimating a "safety index" is readily apparent.

These facts would lead one to suppose that eventually the whole Stegomyia population could be destroyed in a given control area, but this is rarely the case, because of certain conditions that cannot be guarded against. In the first place every inspector, even though he may be a most excellent man, is apt to make mistakes. We know of one director who thinks that every inspector omits an average of 5 per cent of all the breeding-places in his district. This would seem to be a high estimate, for we never like to have a recheck show that an inspector has overlooked more than one breeding-place in a block. And the breeding-places missed are usually not the larger ones, the barrels and tambours, but more often bottles, pitchers, or jars that are hidden away under or in the house, and which, from their very lack of size, are incapable of producing very many adult mosquitoes. These few adults may sooner or later deposit eggs in a water-barrel where the resulting larvae are seen and destroyed by the inspector.

Frequently it happens that where an attempt is made to control breeding with petroleum products, a few larvae prove refractory, so that on resurvey an inspector is apt to find one or two wrigglers in a container. This often occurs in *lejía*, and occasionally a barrel of fresh water is seen with a fairly good covering of oil but with one or two larvae underneath. It should also be noted that the adult Stegomyia is not in the habit of depositing all her eggs at one time or even on the same day. It may thus happen that several containers are contaminated by one mosquito, each deposit having but very few larvae.

Moreover, eggs of the Stegomyia are not always deposited on the surface of the water, but sometimes along the damp edge of the container, and as the water recedes they are left high and dry. Here they may remain quiescent for months, or until the next rain floats them off. Thus it is that after the first rain following a dry period the larval index suddenly increases.

CONCLUSION

From the above considerations it may be seen that a larval index in a city in which active control work is going on has a very different significance from a like index in a city in which no attempt is made at Stegomyia control, and that a larval index in a controlled city is only relative and in no way indicates the true percentage of adult mosquitoes that may emerge. It is of value, however, both to the central office and to the men in the field, as an indication of the progress of the work. Whenever the mosquito index is plotted and conspicuously posted, we have noted that the inspectors have been keenly interested and have made special efforts to secure the lowest index for their districts.

Our experience up to the present time leads us to believe that the deposit index is the generally most useful indicator in yellow-fever control work.

SUMARIO

De acuerdo con las consideraciones arriba expresadas, se verá que el índice larval de una ciudad en donde la campaña en contra de la fiebre amarilla sea activa posee una significación muy diferente al índice de otra ciudad en donde ningún esfuerzo se haya realizado en contra de la Stegomyia. Es cierto que el índice larval de una ciudad bajo restricciones es solamente relativo y de por si no indica el tanto por ciento de mosquitos adultos que puedan brotar. Sin embargo este resulta ser de mucho valor a la oficina central y a los inspectores sanitarios como indicación del progreso del trabajo de eradicación. El fijar carteles conteniendo cuadros gráficos demostrativos del índice de mosquitos desarrolla gran interés por parte de los inspectores y estimula esfuerzos tendentes a disminuir a lo mas mínimo el índice de sus distritos respectivos. Nuestra experiencia actual nos lleva a creer que el índice de depósitos es el indicador de mayor utilidad en las campañas de dominio de la fiebre amarilla.

COMMENT

The following comments on the paper by Drs. Connor and Monroe, written in a letter to Dr. Connor by Dr. H. R. Carter of the United States Public Health Service, are published at Dr. Connor's request and with Dr. Carter's permission:

1. Your division of the infectible area into "endemic" and "epidemic" zones is in accordance with the nomenclature in Lyster's article, signed by Gorgas and myself (1920). As I revised it, naturally I have "no kick coming," yet I cannot help thinking that the division of the infectible territory into three zones,—(*a*) "epidemic," (*b*) "en-

demic," and (*c*) "permanently endemic"—is a better one, the divisions being based on territory alone, as in the following examples:

(*a*) Places like New Orleans, infectible at the proper season, but with the infection limited absolutely by the season, *Stegomyia activity ceasing in cold weather.*

(*b*) Places like Colón and Tampico in which fever on introduction might last one, two, or three years—*Stegomyiae being active the entire year*—and its duration dependent upon the proportion of susceptible human material furnished.

(*c*) Permanently endemic places, like Habana and Rio, in which Stegomyiae are active at all seasons and sufficient susceptible material is steadily furnished to keep up the infection permanently.

2. I do not know that it "has been generally accepted" that babies play a greater part in the continuance of yellow fever than do immigrants. I discussed this matter briefly, as one must in a short article, in my paper on the "Spontaneous elimination of yellow fever, etc." I have never attempted to evaluate numerically the *comparative* total effect of immigration and birth. Usually it is the large cities, which naturally have many births, that have a large immigration, and so both factors increase together. I am sure that immigration is much the more important in a focus small in *area*, as every city is; while in a large *regional focus*, such as that of the south shore of the Caribbean and the lesser Antilles, the children were the more important factor.

3. Exception might be taken, however, to your implication that there is little or no relation between the birth-rate and the number of yellow-fever cases. As you know, cases are not reported among natives —certainly not among native children in places where yellow fever is permanently endemic—and yet I think you will agree that everyone living in such a focus for X years becomes immune to yellow fever and that he becomes immune only by attack. Obviously, then, to your reported cases of yellow fever you must add for each year *on the average* a number approximately equal to the annual number of births. I say "approximately equal" because, of course, a certain number of children die before they are immunized, but all who live X years have had yellow fever. This number of cases of yellow fever then *has* been occurring in households the elder members of which are immune. The fact of their not being reported—none of them are—does not affect their infection of Stegomyiae.

During two years in Habana, although the Yellow Fever Board was supposed to see every case *suspected* of being yellow fever (and *did* see every case reported as such, and many others), only a single Cuban child was brought to our attention, and this one had measles. Yet there were 4000 to 6000 births per annum in Habana.

4. I like your expression "hacienda endemic center." Also I like your discussion of it. If, however, you consider a city as being *not a unit,* but a number of units in more or less close relation with each other and if you consider the regional foci, like the south shore of the Caribbean and the lesser Antilles, I think you can find rather close analogy in the laws governing continuance in all of them. We have never found all parts of a city—a permanently endemic focus—infected at the same time. It spreads from one house to another, from one barrio to another—not always to contiguous places, because people frequently do not develop a disease where it is contracted, or even close to that place.

5. In some regions, even where the labor is not indentured, there is little travel (which is the essential) from place to place. In such cases elimination by failure of the human host is usually quite certain: Porto Rico is such an instance as, I think, were the French Antilles. Guiteras classes such places as with "stagnating travel."

6. You are right in saying there are not many true permanently endemic hacienda infections reported. That of Yucatán and Campeche —the one I first used for illustration in 1914—is the best known. Possibly the same existed about Coro, Venezuela (sugar), and I certainly would not have trusted the sugar district around Trujillo, Peru, to have cleared itself spontaneously—especially in conjunction with the rice haciendas north of it.

7. Your discussion of the different Stegomyia indices is clear and good. I do not know that the index of containers "is the most valuable." If it be to measure *the carefulness of the work done by your inspectors*, it is. If, however, it is for the purpose of measuring the number of Stegomyia breeding-places to the population, your house index is best.

I think that the house index—having made proper allowance to determine the emergence of imagoes in it—is most useful in determining *the value of the work done*—that is, how close we are coming to reducing Stegomyia below the limit at which the fever can be conveyed. This (as you very justly say) should be taken for the different sections of the town—the critical index for each section varying inversely with the proportion of susceptible people in such section.

8. Your point that during a Stegomyia campaign the index of breeding is not the index of production (of imagoes) is extremely well taken. For some time at least the first will depend upon the number of Stegomyiae in flight and their access to containers; the second upon the carefulness of your inspector. Where you use fish, however, the first part of this statement does not hold. You may have noticed that in the report on Peru we mentioned "small larvae, large larvae, pupae." Obviously, the presence of small-sized larvae means *nothing* in the way of production.

9. Your distinction between the parts of a city where immigrants and tourists congregate and the sections occupied by older residents or immunes, is particularly well taken. It means that a city is to be considered epidemiologically as a number of units—not simply a single one. You must remember, however, that although all reported cases occur where strangers live, yet if the indigenes are being immunized, yellow fever *must* be occurring among them also, although unreported.

I do not know that it is also true that there are more water-containers around hotels in proportion to the number of people than in private houses. I suspect, however, that this is usually the case.

10. Have you ever used what Howlett calls "trap breeding-places?" They were first used by Murphy, Le Prince, and myself in Panama in 1905. The breeding-places of election being destroyed, the Stegomyia will be seeking others—usually more difficult to find. Put a couple of calabashes or a *tinaja* in a dark and shady place for her to lay in, which your inspector can turn over weekly. She will not seek the out-of-the-way places.

11. Stegomyia larvae will live quite a while under a film of petroleum —that is, one to three days. Pupae fortunately will not, but I count petroleum as of *very* doubtful efficiency in a campaign against Stegomyiae.

NOTES ON THE LIMIT OF THE USEFULNESS OF FISH IN LARVAE (AEDES CALOPUS) CONTROL

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Present day methods of attacking yellow fever are based on the control of the insect carrier during its aquatic cycle. The introduction of the use of fish in fresh water containers for this purpose has caused a veritable revolution, and several recent demonstrations of control have been successful mainly through the continued use of several well chosen varieties. These are used in barrels and in cisterns built under the surface of the ground. They will not as a rule live in metal tanks, or metal cisterns, or in water too strongly alkaline, or in collections of water too small to support fish life.

The fish used may for general purposes be divided into two main groups; the "top feeders" and the "bottom feeders." At first the top feeders were used in water barrels and fountains to which the sun had free access, leaving the bottom feeders to be used in underground cisterns, and in other deposits to which the sun was not freely admitted. Soon, however, it was found that the bottom feeder could and would live in the light as well as in the dark, so that lately they have the preference for all deposits. They are as a class more hardy, and their position near the bottom makes them less liable to accident, and gives them an advantage, as the mosquito larvae are essentially bottom feeding insects. This is an important factor in large containers, since the "top feeders" will not continue active if forced to hunt their prey at a depth. Some cisterns are six feet or more deep.

The number of fish used has varied greatly but by common practice a single bottom fish, and preferably a male, is now placed in a cistern and a pair in a barrel. More could be used, but their excretions might be unwelcome and so cause them all to be destroyed. In normal times and under average conditions two fishes should be sufficient, but occasionally one finds deposits, barrels especially, with a pair of fine fish in which there are uncountable larvae. This happened so frequently in a city in which I recently worked that I decided to look into it more closely, carrying out some experiments to determine the larvae destroying capacity of fish, and correlating this with any physical characters the city might present.

In the first place, the city water supply comes from a municipally owned plant designed for a population of 45,000 and is therefore quite inadequate for a population of 100,000 to which size the city has increased in the past two or three years. During the past year city water has been available at best only a few hours each day. Frequently for periods of two or three weeks there has been no water at all. With the installation of new pumps a continuous and adequate supply recently became available both night and day. Remembering former lapses, the people were loath to give up their water barrels, and continued to keep them full of water although they were no longer in use. It thus happened that the cleanliness of the family barrel was not now a thing of first importance to the housewife. If the sanitary inspector knows that the barrel has had fish in it, or is assured by the lady of the house that it has, he is apt to hasten his rounds by neglecting to look into it. On the second or third tour of inspection he may examine it more closely only to find it actually swarming with larvae in spite of the pair of fish that may appear to be in perfect health. This is the usual occurrence, for one never sees a barrel supporting a *few* larvae if there are fish in it; there are always countless numbers.

To determine the number of larvae that a fish can actually destroy, we kept two in separate buckets in the office, providing them at all times with an excess of fourth stage Stegomyia larvae. For a period of one week a record was kept of the daily average number of larvae that each one ate. One of these was a bottom feeder, *Dormitator maculatus*, and the other a top feeder, *Gambusia nicaraguensis*. Both were about equal size, and as nearly as possible like those we use in our work. These two fish had had a maximum feeding a month previously, but since that time had been in their respective buckets without other food than what they could secure from the water they were in. During the subsequent week's feeding they were found to be capable of destroying an average of 161 larvae per day, or, in round numbers, 150.

Allowing a ten day period for the changes from egg to adult, each fish in that time would consume 1500 larvae, and a pair of fishes 3000. Assuming that each female mosquito deposits an average of 200 eggs, it will be seen that the two fish would take care of all larvae resulting from the eggs of fifteen female mosquitoes or less. An average of 200 eggs per female for Stegomyiae is too high, but as there will also be Culices in the same barrel, and as the number of their eggs is usually as much above 200 as the Stegomyia total is below, 200 would seem to be a fair average.

If more than fifteen females deposit their eggs in the barrel and the larvae from them are missed by the inspector for a cycle or two, the results are very striking. Let us suppose that twenty females have deposited 4000 eggs in a certain barrel. By the end of ten days the fish would have accounted for 3000 of them. Of the remaining 1000, 500 would be females, and of these it would be fair to assume that half, or 250, would be able to secure a blood meal and later deposit eggs. The resulting larvae would total 50,000, of which the fish would have consumed 3000, leaving 47,000 to reach the adult stage. The next generation, assuming the same proportion of females, and of casualties, would amount to the formidable total of 2,400,000 of which the fish could destroy only 3000. Before this number had been reached, however, the capacity of the water in the barrel to support larval life would have been surpassed, and many larvae would have perished from lack of food or air. To the inspector seeing the barrel it would appear that the fish were living along with the larvae in closest friendship, and making no attempt whatsoever to destroy them. It is not to be wondered at therefore, that remarks are sometimes heard that fish control of larval breeding is not always to be relied on. On the other hand, the more enthusiastic of the directors explain this apparent delinquency of the fish by saying that sometimes fish get stale and need to be changed.

In order to determine whether or not a fish would get stale, I had three fish brought into the office, one from each of three barrels where they were living with countless numbers of larvae, together with some of the larvae from the same containers. These fish ate not only an average number of larvae while under observation, but larvae from the same barrel that the fish had been in before. Unquestionably these fish were not stale; rather they had had so much to eat that they could not make any impression on the huge number of larvae.

Generally it may be said that larvae will be found in barrels in which there are a pair of fish when the expression n (200) − 3000 is greater than one, n representing the number of females depositing eggs. Assuming that n (200) −3000 is greater than one, then if the barrel should remain undisturbed for two or more cycles, the maximum number of mosquitoes would correspond to the formula:

$$\frac{(n\,(200) - 3000)\;\overline{200}}{(4)\qquad\qquad 4}\,\overline{(-3000)}^{\,x-1}$$

where x equals the number of cycles taken into account.

It will be noted that this only shows the mechanism by which it is possible to find larvae and fish co-existing, and it should not be inferred that this is a frequent occurrence, for it is not. Perhaps only 1 or 2 per cent of all barrels inspected will be found harboring both fish and larvae, for where control work is carried on thoroughly, only very rarely will fifteen or more females be on hand to infest a single barrel.

The actual number of larvae destroyed daily by the two fish is given in the following table. The word destroyed is used advisedly for it was noted in several instances that the Gambusia would regurgitate many of the larvae eaten, but these would be dead, nor would the Gambusia touch them again in the presence of living larvae.

	DORMITATOR MACULATUS	GAMBUSIA NICARAGUENSIS
First day	176	185
Second day	146	196
Third day	145	180
Fourth day	150	200
Fifth day	190	210
Sixth day	70	80
Seventh day	145	180
Total	1,022	1,231
Average per day	146	176

These fish were of the size usually used for barrels, but *G. nicaraguensis* is a much heavier fish for its length than *Dormitator maculatus*, so that its efficiency per gram of body weight is much less than for *Dormitator maculatus*. This is shown by the following table.

	WEIGHT	CONSUMPTION OF LARVAE			
		Weekly	Daily	10 day cycle	Per gram of weight per day
	grams				
Dormitator maculatus	2.4	1022	146	1460	60.8
Gambusia nicaraguensis	5.5	1231	176	1760	32.0

CONCLUSIONS

1. An average larvae consumption for the class of fish generally used is about 150 per day.

2. Bottom feeding fish are rather more efficient for their weight than top feeders.

3. When breeding is found in barrels in which there are fish it is not because of the refusal of the fish to eat, but because of (a mathematical factor that places the fish at a great disadvantage in) the presence of a number of larvae beyond the limit of its possibilities to consume.

RESUMEN

El número de pececillos que se usan varía grandemente pero la práctica común es el colocar un pececillo ''de fondo,'' preferiblemente macho, en las cisternas y un par de los mismos en los barriles. Con tal que todo marche normalmente dos peces son suficientes; sin embargo, a veces se encuentran depósitos, especialmente en los barriles, los cuales son grandes criaderos de larvas a pesar de la presencia de los peces. La frecuencia de este hecho nos ha estimulado a llevar a cabo experimentos para determinar la capacidad destructora de un pececillo y correlacionar esta observación con los caracteres físicos del medio ambiente. Con tal objeto mantuvimos dos peces (*Dormitator maculatus* y *Gambusia nicaraguensis*) en receptáculos separados y les proveímos constantemente con un exceso de larvas de Stegomyia. Se tomó nota exacta durante una semana del número de larvas destruídas. Nuestras conclusiones, según lo demuestra el proceso de computación detallado en el informe que precede, son:

1. El promedio, de consumo de larvas para esta clase de peces es de 150 por dia.
2. Los peces "de fondo" son mas eficientes que los "de superficie."
3. Cuando a pesar de la presencia de los peces los barriles resulten ser criaderos de larvas, no se debe esto a que los peces rehusen comer sino a un factor matemático que coloca a los peces en situación des ventajosa y por el cual el numero de larvas alcanza un límite lejos de la posibilidad de destrucción.

THE HEPATIC LESIONS OF EXPERIMENTAL YELLOW FEVER

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When Noguchi, in 1919 (1), published his first pathological findings in experimental yellow fever, he spoke of yellow livers (in which fatty degeneration may be presumed to exist), but in treating of the pathological histology of such organs he described only tumefaction, vacuolization,[1] and necrosis of the cells, the frequent presence of karyokinetic nuclear figures (this phenomenon, which also interested Martin and Pettit (2) in their study of the livers of guinea pigs dying of infection with *Leptospira icterohaemorrhagiae,* is demonstrable also in human yellow fever (3)), the existence of irregularly distributed hemorrhagic foci and periportal lymphocytic infiltration, and finally the presence of *Leptospira icteroides.*

Villamil Mendoza, who infected (in Mérida) four guinea pigs and two dogs with the blood of a guinea pig which had in turn been infected by the injection of blood from human yellow fever, refers, in speaking of the liver lesions in both dogs, to the existence of a "very marked fatty degeneration," a liver like butter (4). With regard to the guinea pigs, he speaks only of friable, slightly yellow livers, with punctate hyperemic areas and hemorrhagic foci.

Perez Grovas, who isolated *Leptospira icteroides* in Vera Cruz by inoculating guinea pigs with the blood of yellow fever patients, says in his description of the autopsy findings in experimental animals: "The parenchyma of the liver revealed fatty degeneration of all the cellular elements, particularly in the region of the blood vessels," (5) and in his conclusions he remarks that very rarely has an experimental disease so similar to the natural been observed.

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[1] As is well known, if the special histochemical technique for the staining of fat is not employed, the cells which are attacked by a distrophic steatosis may appear as simply vacuolated.

Finally, Garcia Rendon (6) describes fatty degeneration of the parenchyma of the principal organs, most notable in the liver.

The Cuban investigators (Lebredo) began their first discussion with regard to the etiological relation of *Leptospira icteroides* to yellow fever (7) immediately after the appearance of Noguchi's preliminary report (8) and before any experimental details had been published. Later when a strain of *Leptospira icteroides* had been placed at their disposal, they (Guiteras, Lebredo, and Hoffmann), claimed in support of their position the alleged absence in animals experimentally inoculated with *Leptospira icteroides* of the lesions characteristic of the human disease, principally the gastric and hepatic lesions. Guiteras (9), ex-Secretary of the Cuban Department of Health, made the statement before the Academy of Medical Science of Havana in the session of February 14, 1921, that the anatomical lesions produced by *Leptospira icteroides* are very different from those encountered in human beings who have died of yellow fever and are identical with those of infectious jaundice. Lebredo reported that he had not encountered in his experimental studies the typical gastric hemorrhages and fatty degeneration observed in human cases (10, 11) and W. H. Hoffmann, working with tissues obtained by Lebredo in the Havana laboratory from animals experimentally infected with the Mérida strain of *Leptospira icteroides*, stated that he had not encountered necrosis or fatty degeneration of the hepatic parenchyma in a single instance, and that "always and without exception the nuclei of the hepatic cells was everywhere well stained" (12).

The conflicting histopathological findings led me to undertake a histopathological study of the livers of guinea pigs infected with the strain of *Leptospira icteroides* isolated in Veracruz by Perez Grovas. The following is a brief account of my findings.

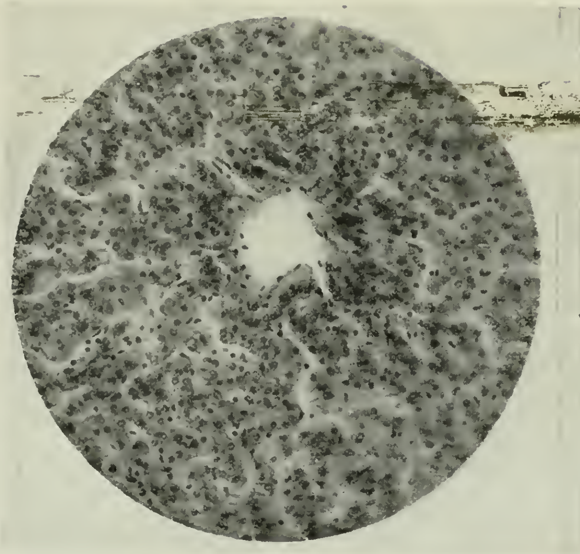

Fig. 1. Normal Human Liver for Comparison
Exsanguinated hepatic vein

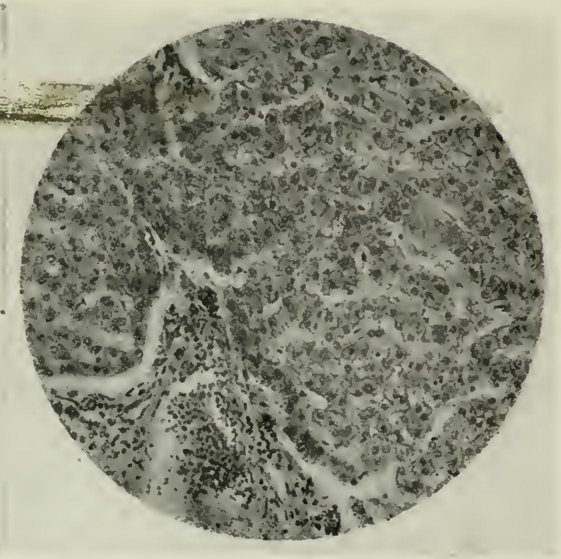

Fig. 2. Normal Human Liver for Comparison
Portal vein moderately congested

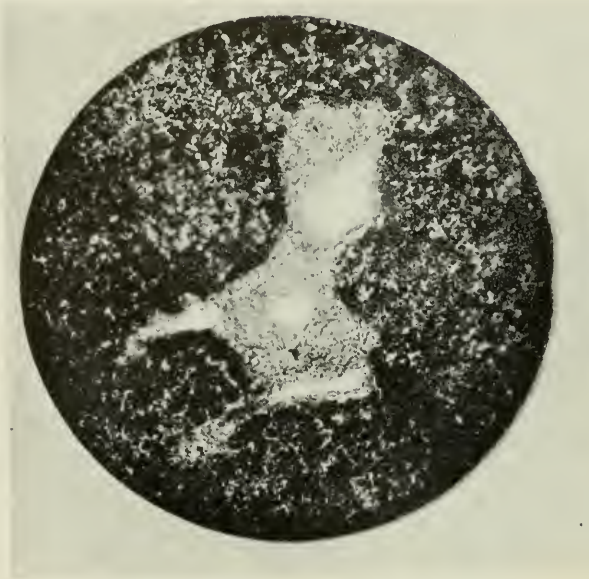

FIG. 3. LIVER FROM HUMAN YELLOW FEVER

Fatty degeneration in the periphery of the lobules. Exsanguinated portal vein.

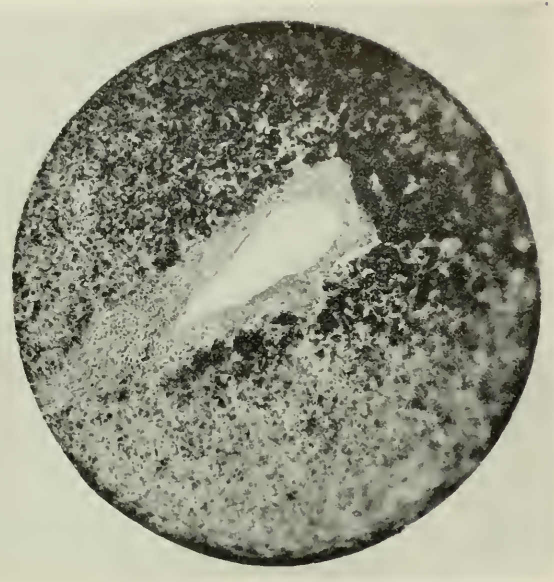

FIG. 4. LIVER FROM HUMAN YELLOW FEVER

Fatty degeneration limited almost exclusively to the periphery of the lobules. Exsanguinated portal vein.

Previous study of the rather abundant human yellow fever material which I have had at my disposal in past years had demonstrated the frequent coexistence of necrotic lesions and phenomena of fatty degeneration in the liver. The predilection of the latter for the periphery of the lobules (figs. 3 and 4) is in accordance with Manson's results (13) and contrary to those of Sanarelli (14). In the predominance of hepatic congestive lesions over portal (fig. 5) my findings also differ from those of Sanarelli.

In the guinea pig dying of infection with *Leptospira icteroides* the hepatic lesions which we encounter, aside from the congestive and the hemorrhagic, are of two kinds. They do not differ in nature from those presented by animals succumbing to experimental infectious jaundice, but they differ, and notably, in their intensity. On the other hand, neither in disposition, extent, nor severity can the hepatic lesions of experimental yellow fever in the guinea pig be differentiated from those of human yellow fever. The two types of lesions demonstrated are (1) profuse fatty degeneration and (2) insular necrosis, nor does the latter proceed from ostensible degenerative phenomena. The former, a distrophic steatosis of acute course, may appear with an intensity never observed in infectious jaundice, human or experimental, nor even in phosphorus poisoning in the guinea pig, and it is altogether comparable with that occurring in yellow fever in man. The lesion affects extensive regions, without any evident predilection for a given hepatic zone, and it may affect the whole lobule, may be most intense in the periphery (fig. 6), or may be distributed in broad triangular areas with the base at the periphery and the point of departure apparently the central vein (fig. 7). As in human yellow fever, central, intralobular or hepatic congestion (fig. 7) predominates over peripheral, interlobular, or portal (fig. 6).

The insular necrosis may be limited to part of a lobule, to a single whole lobule, or may affect several (figs. 8 and 9). Through the necrotic masses, the sinusoids preserve their permeability, carrying compact rows of neutrophilic polynuclears. In fact, these lesions are similar in nature to those of the guinea pig in-

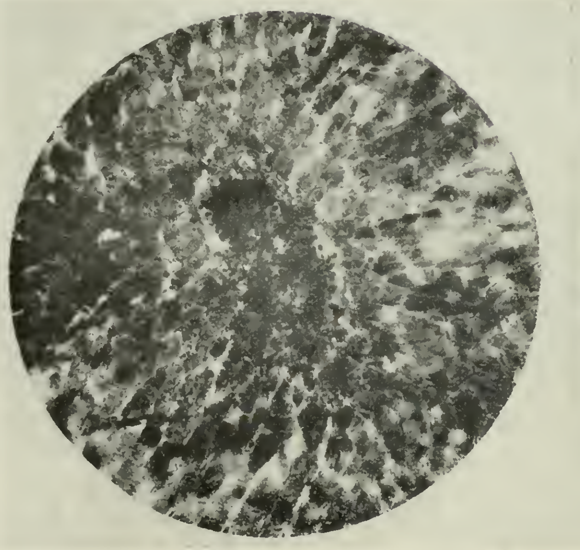

FIG. 5. LIVER FROM HUMAN YELLOW FEVER

Intralobular congestion

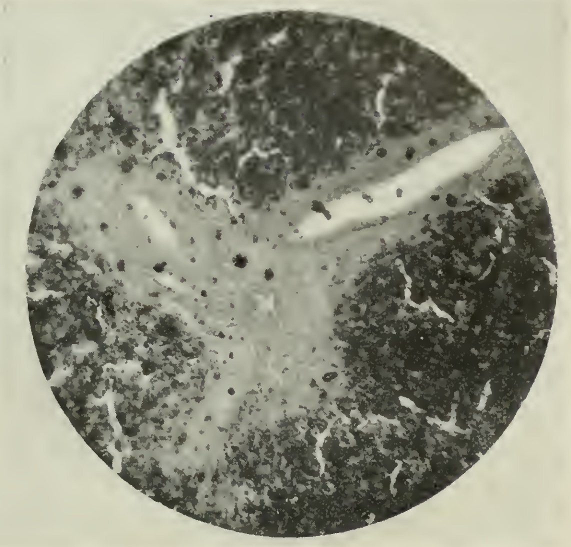

FIG. 6. LIVER OF GUINEA PIG INFECTED WITH LEPTOSPIRA ICTEROIDES

Intense and profuse fatty degeneration. Peripheral region of the lobules.

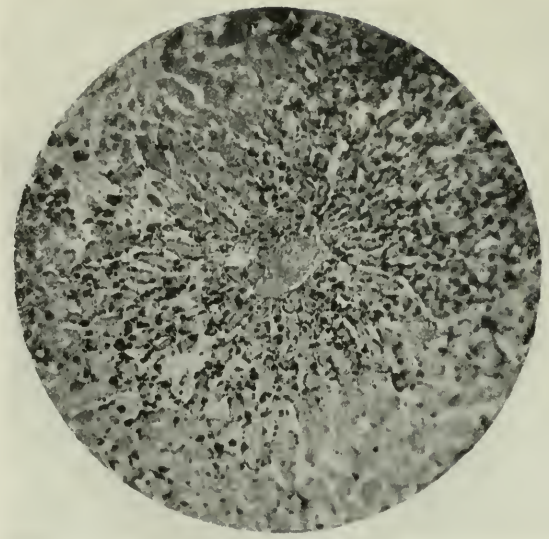

FIG. 7. LIVER OF GUINEA PIG INFECTED WITH LEPTOSPIRA ICTEROIDES

Fatty degeneration. Central region of a hepatic lobule.

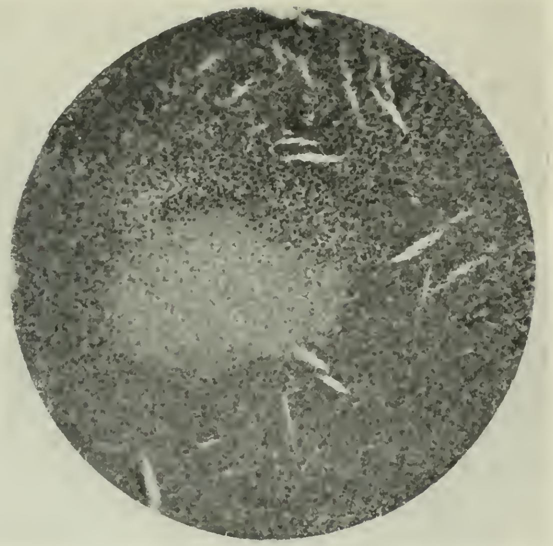

FIG. 8. LIVER OF GUINEA PIG INFECTED WITH LEPTOSPIRA ICTEROIDES

Small focus of insular necrosis

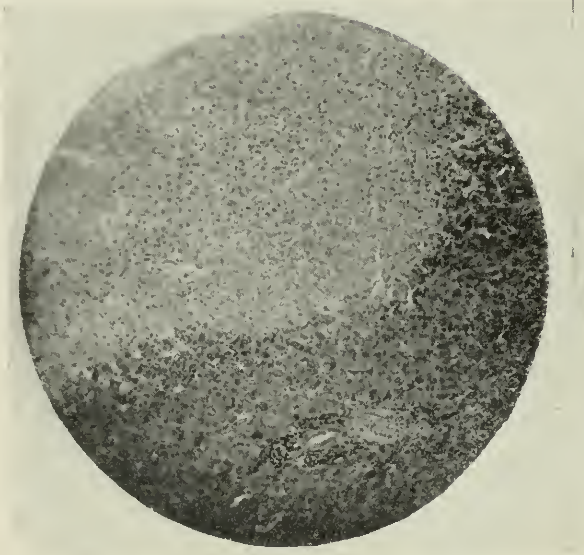

Fig. 9. Liver of Guinea Pig Infected with Leptospira icteroides

Extensive area of insular necrosis

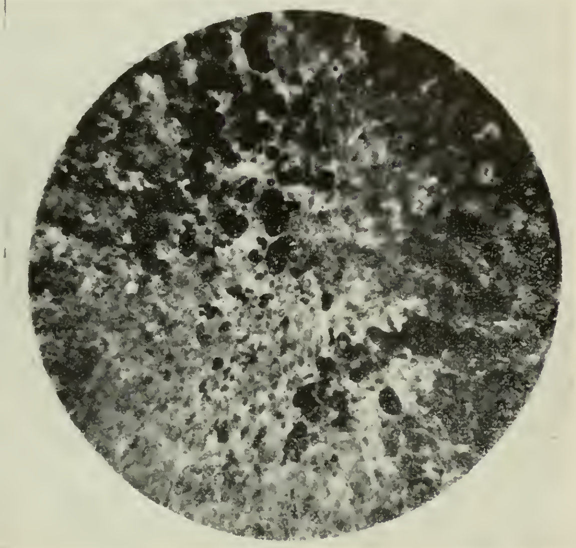

Fig. 10. Liver of Guinea Pig Infected with Leptospira icteroides

Fatty degeneration with foci of insular necrosis fected with *Leptospira icterohaemorrhagiae*, except for their greater extent and for the invasion of the parenchyma by accumulated leucocytes of neutrophilic type; in livers with necrotic foci from experimental infectious jaundice it is exceptional, according to Martin and Pettit (15), to find neutrophilic polymorphonuclear leucocytes in the parenchyma.

Finally, the frequent association of the lesions described (fig. 10) is noteworthy. Foci of fatty degeneration alternate with wide necrotic zones, bordering on them in some regions.

The parenchymatous architecture of the liver usually persists, as in human livers, notwithstanding intense phenomena of degeneration (fig. 7), but it may be totally destroyed by the degenerative process, as in the specimen shown in figure 6, from a preparation recently sent by Dr. Noguchi to Dr. Vasconcelos. In the spaces of Kiernan the hyperplased fibrous stroma protects the hepatic ducts, which in general seem to be little altered. Not so the blood vessels, for the portal veins, as well as the hepatic arteries, usually present lesions of endo-and perivascularitis. We have not observed phenomena of defensive reaction in the cells of Kuppfer; rather, the considerable infiltration of leucocytes in the sinusoids appears to indicate that to these belongs the principal phagocytic activity.

DISCUSSION

The results of the present investigation are in agreement with those of Noguchi, Villamil Mendoza, Perez Grovas, and Garcia Rendon in demonstrating the similarity of the lesions produced in the guinea pig by inoculation of *Leptospira iceteroides* to those of human yellow fever.

Although the materials used by Guiteras, Lebredo, and Hoffmann were tissues derived chiefly from guinea pigs inoculated by Lebredo with a strain of *Leptospira icteroides* isolated by Noguchi and Kligler in Mérida, yet careful examination of the experimental data given by Lebredo (10) indicates possible intervention by an intercurrent bacterial infection in his guinea pigs. The incubation period for *Leptospira icteroïdes* infection in guinea pigs, as recorded by Noguchi (12) and others (6), is never shorter than forty-eight hours and varies between three and six days in the majority of instances. Yet 70 per cent of Lebredo's animals registered a rise of temperature (to 40°C.) within thirty-six hours after inoculation, and in 17 per cent of his animals there was no incubation period at all, or at most a few hours. A fever rising to 40°C. within twenty-four to thirty-six hours and remaining high for the following days constitutes reliable evidence of the presence of secondary infection (16). Such an infection would of course interfere with the normal course of the *icteroides* infection, and organs taken from such animals would not be likely to show the pathological changes characteristic of a pure infection with *Leptospira icteroides*.

CONCLUSIONS

In the livers of guinea pigs which died after injection of virulent cultures of *Leptospira icteroides* there has been found (apart from intense congestive and hemorrhagic phenomena, fibrous reactions of the stroma and vascular walls, etc.) two principal types of lesions: fatty degeneration or distrophic steatosis, acute and profuse, and insular necrosis.

The fatty degeneration affecting the whole protoplasm and the nucleus of the hepatic cells and destroying extensive regions of the glandular parenchyma has never been described either in human or in experimental infectious jaundice; on the other hand, it is analogous to that found in human livers from fatal cases of yellow fever.

The insular necrosis presents characteristics analogous to those observed in experimental infectious jaundice in the guinea pig.

The hepatic histopathology of the guinea pig in *Leptospira icteroides* infection cannot be used as an argument against the etiologic significance of that organism in yellow fever. On the contrary, the statement made by Perez Grovas has been confirmed; namely, that the experimental infection incited in the guinea pig by this organism is analogous in its symptoms and lesions to the natural disease in man, and that rarely is an experimental disease observed which is so similar to the natural (7).

CONCLUSIONES

En los hígados de cuyes muertos por inyección de cultivol virulentos de leptospiras de Noguchi encontramos (aparte intensos fenómenos congestivos y hemorrágicos reacciones fibrosas del estroma y de las paredes vasculares, etc.) dos principales tipos de lesiones: la degeneración grasienta o esteatosis distrófica, aguda y la necrosis insular.

La degeneración grasienta afectando a la totalidad del protoplasma y nucleo de la celula hepática y destruyendo extensas regiones del parenquima glandular, nunca se ha descrito en la espiroquetosis ictero-hemorrágica humana, ni en la experimental; siendo análogo, en cambio, a la que presentan los higados humanos en los casos mortales de fiebre amarilla.

La necrosis insular presenta caracteres análogos a los que se observan en la espiroquetosis ictero-hemorrágica experimental en el cuy.

La histo-patologia hepática del cuy, en la leptospirosis de Noguchi, no puede utilizarse como argumento en contra del valor etiológico del espirogermen icteroides. Antes por el contrario, robustece las afirmaciones de Perez Grovas: La enfermedad experimental provocada en el cobaya es analoga, en sus sintomas y lesiones, a la enfermedad natural en el hombre. Muy rara vez se observa una enfermedad experimental que sea tan semejante a la natural.

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TRANSMISSION OF ROCKY MOUNTAIN SPOTTED FEVER BY THE RABBIT TICK HAEMAPHYSALIS LEPORISPALUSTRIS PACKARD

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It has been suggested by the writer in previous papers that the wild rabbits of Montana—jack, cottontail and snowshoe rabbits—are possible factors in the maintenance in nature of the virus of Rocky Mountain spotted fever (1, 2, 3,). This suggestion has been the result of extended field studies, especially in Eastern Montana. In this connection the importance of demonstrating whether or not the rabbit tick, *Haemaphysalis leporis-palustris* Packard, is capable of transmitting the infection has been apparent for several years. It has been evident that if this tick can transmit spotted fever infection, then these native species of rabbits would be hosts of two ticks capable of transmitting the fever: one, the rabbit tick which would probably transmit the infection only among rabbits (the possibility of its occurrence on birds must not be forgotten, although their susceptibility to spotted fever seems unlikely); the other, the so-called Rocky Mountain spotted fever tick, *Dermacentor venustus* Banks, which utilizes not only rabbits but many other rodents as its hosts and is the tick which transmits the infection to man. Not until 1921, however, did it become possible to undertake the study of the infectivity of this tick and it is the purpose of the paper to present the results of a few selected experiments which demonstrate, first, that the rabbit tick can transmit spotted fever from rabbit to rabbit; second, that infected rabbit ticks occur in nature and third, that the infection may be present in eggs deposited by infected females.

In the experiments about to be outlined the rabbit ticks were fed under brass gauze capsules secured by adhesive tape to the bellies of the rabbits used. The hair was removed by shaving but was left clipped short where the ticks were to feed, under the dome-like portion of the capsule. A non-irritating soap was found essential for shaving, both to avoid undue irritation to the delicate skin of the rabbits and in order not to repel the ticks and prevent them from feeding.

Since Rocky Mountain spotted fever often runs a mild course in rabbits and is frequently difficult or impossible to diagnose with certainty, blood transfers to guinea pigs were relied upon for diagnosis whenever external lesions were absent or not sufficiently marked. Whenever uncertainty existed concerning the results of transfers to guinea pigs, immunity tests were given.

EXPERIMENT DEMONSTRATING THE TRANSMISSION OF ROCKY MOUNTAIN SPOTTED FEVER FROM BELGIAN TO BELGIAN BY MEANS OF THE RABBIT TICK (HAEMAPHYSALIS INFECTIVITY EXPERIMENT 2)

April 21. Belgian 2 inoculated with 1 cc. citrated blood from patient C. This rabbit ran a typical course of spotted fever and recovered.

Temperature record of Belgian 2

April 22	102.0	May 1	104.8
April 23	102.0	May 2	104.2
April 24	102.0	May 3	103.8
April 25	102.4	May 4	103.4
April 26	102.2	May 5	102.8
April 27	103.8	May 6	102.6
April 28	104.6	May 7	102.8
April 29	105.4	May 8	102.2
April 30	105.0	May 9	102.2

April 27. A number of *H. leporis-palustris*, Lot 22-1, secured from snowshoe rabbit 22, placed on Belgian 2, in brass gauze capsule.

April 30. The above rabbit ticks removed from Belgian 2, and kept in pill box over moist sand. Some had been observed to feed.

May 3. Ticks removed from pill box and placed in capsule on normal Belgian 3, which ran a typical course of spotted fever and recovered.

Temperature record of Belgian 3

May 4	101.8	May 13	102.6
May 5	102.6	May 14	103.2
May 6	102.2	May 15	102.8
May 7	102.0	May 16	102.2
May 8	102.6	May 17	102.6
May 9	106.0	May 18	103.0
May 10	106.6	May 19	103.0
May 11	104.0	May 20	102.8
May 12	104.4	May 21	102.4

May 9. Rabbit ticks removed; 12 alive; 9 feeding; several dead.

May 10. Three cubic centimeters citrated heart's blood from Belgian 3, inoculated in Pig 44, which ran a definite but mild course of spotted fever and recovered.

Temperature record of Pig 44

May 11	102.0	May 19	105.0
May 12	101.6	May 20	105.4
May 13	102.8	May 21	105.0
May 14	102.6	May 22	103.4
May 15	102.0	May 23	104.0
May 16	102.6	May 24	103.2
May 17	103.4	May 25	102.8
May 18	104.0	May 26	102.6

This experiment, the first tried, seems to demonstrate conclusively the ability of the rabbit tick to transmit spotted fever from Belgian to Belgian. It could be objected that the ticks used might have been infected before feeding on Belgian 2, that is, in nature. This is certainly true, but if so, it simply means that an infection acquired from some rabbit in nature was transmitted to Belgian 3, and in no way detracts from the conclusions.

EXPERIMENT DEMONSTRATING THAT INFECTED RABBIT TICKS EXIST IN NATURE (HAEMAPHYSALIS INFECTIVITY EXPERIMENT 8)

May 28. Twenty *H. leporis-palustris* (18♂, 2♀, Lot 108-c-1) from wild snowshoe rabbit 108-c, attached in capsule to Belgian 8.

June 3. Ticks removed; 12 alive; 9 feeding; 8 dead.

June 10. Pig 79 inoculated with 1 cc. citrated heart's blood from Belgian 8.

The temperature of Belgian 8 was taken each morning and usually each evening until July 23, a period of fifty-six days. After the seventh day the afternoon temperature varied between 103.2 and 104 until the fiftieth day. This record was not different, however, from that frequently found in normal rabbits, nor were there any apparent external lesions of spotted fever.

Pig 79 was inoculated from Belgian 8 on the thirteenth day after the ticks were placed on the rabbit. This pig ran a mild but typical course of spotted fever and recovered.

Temperature record of Pig 79

June 11	102.4	June 18	103.8
June 12	102.0	June 19	103.6
June 13	102.0	June 20	102.4
June 14	101.8	June 21	102.0
June 15	102.8	June 22	101.8
June 16	104.2	June 23	101.4
June 17	105.6	June 24	101.8

The above experiment was the most definite of several of a similar character and showed beyond reasonable question that infected rabbit ticks exist in nature. In Belgian 8, the disease could not be recognized, a condition frequently encountered in working with rabbits as with other wild rodents. In this case, however, such definite results were secured by the blood transfer to pig 79, that no chance for doubt existed.

EXPERIMENT DEMONSTRATING INFECTIVITY OF RABBIT TICK EGGS (HAEMAPHYSALIS EGG INFECTIVITY EXPERIMENT 1)

June 30. Two hundred eggs deposited by female rabbit ticks from Lot 22-1, and which fed on Belgians 2, 3, and 4, crushed and inoculated into pig 95. Eggs seemed somewhat dry.

Temperature record of pig 95

July 1	103.6	July 8	101.4
July 2	102.2	July 9	101.2
July 3	102.6	July 10	100.8
July 4	102.6	July 11	100.8
July 5	102.6	July 12	101.8
July 6	102.2	July 13	101.8
July 7	103.6		

Pig 95 showed no evidence of spotted fever and on October 11, one hundred and three days later, was given an immunity test consisting of 1 cc. of virus of a very virulent strain from pig 154.

Temperature record of Pig 95 following immunity test

October 12	102.2	October 17	102.6
October 13	102.2	October 18	102.6
October 14	102.6	October 19	102.6
October 15	103.2	October 20	102.2
October 16	103.2		

Pig 95 showed no evidence of spotted fever from the immunity test indicating that it had been immunized by the inoculation of tick eggs, the latter therefore being infected.

This experiment indicates that infected females may transmit the virus to the egg as in the case of the fever tick. It is probable that stage to stage transmission likewise occurs. The eggs used in this experiment were deposited by females used to transmit infection from Belgian 2 to Belgian 3 in *Haemaphysalis* infectivity experiment 2, the first experiment noted above.

SOME REMARKS ON THE SIGNIFICANCE OF THE TRANSMISSION OF ROCKY MOUNTAIN SPOTTED FEVER BY THE RABBIT TICK

It is not possible at this time, nor is it advisable to attempt, to point out the full significance of the transmission of Rocky Mountain spotted fever by the rabbit tick. Its relationship to the maintenance of the disease in nature might be easily overestimated or perhaps underestimated. Some possibilities are so apparent, however, that they seem worth noting.

1. It seems probable that Rocky Mountain spotted fever can be maintained among wild rabbits by means of the rabbit tick without the presence of the wood-tick, *Dermacentor venustus* Banks.
2. It is furthermore probable that this fever may exist in the rabbit population in localities in which human cases have not been known and also that it may be far more generally distributed among the rodents in the northwestern states, especially in rabbits, than the known distribution of human cases would indicate.

3. The data involving the rabbit tick indicate that in seeking for the factors responsible for the maintenance of Rocky Mountain spotted fever in nature, rabbits must be reckoned as one of the possibilities. The importance of this rabbit factor, however, is yet to be determined. Indications point to a possible fundamental importance in the type of country where rabbits are, periodically at least, the dominant species of rodent—for example, under strictly prairie conditions. Under mountain conditions such as exist in the Bitter Root Valley, however, with a more diversified rodent population, with more species of susceptible rodents and with more varied habitat conditions, the value of this rabbit factor is less evident.

SUMMARIO

El autor hace mención de pasadas publicaciones en donde ha sugerido la importancia de las varias especies de conejos de Montana como posibles factores en la diseminación del virus de la Fiebre de las Montañas Rocallosas. Parece, nos dice, que el conejo puede ser huesped de dos variedades de garrapatas capaces de transmitir la infección, una de ellas (*Dermacentor venustus Banks*) que utiliza además del conejo otros roedores y la pasa directamente al hombre, y la otra (*Haemaphysalis leporis-palustris Packard*) que la transmite de un conejo a orto. Esta última especie ha sido objeto de investigación en esta ocasión y después de exponer sus experimentos llega a las siguientes conclusiones:

1. Que es probable que la Fiebre de las Montañas Rocallosas pueda ser mantenida entre los conejos silvestres por medio de la garrapata de conejo misma sin intervención de la garrapata de bosque (*Dermacentor venustus Banks*).

2. Es mas probable aún que la fiebre pueda existir entre la población de conejos en localidades en donde ninguna infección humana haya existido y que asi mismo puede que esté mayor distribuída entre los roedores de la región noroeste que lo que la distribución entre humanos podría indicar.

3. Los datos acerca de la garrapata del conejo indican que al buscar los factores responsables del mantenimiento de la enfermedad en estado natural, estos animales deben considerarse como

una de las posibilidades. La importancia del conejo en este sentido tiene aún que determinarse definitivamente. Hay indicaciones de fundamental importancia en el terreno de aquella clase de región puramente de praderas, en donde, por lo menos periódicamente, estos animales constituyen la especie dominante. Las regiones montañosas, como el valle del Bitter Root, en donde hay variedad de especies, susceptibilidad y habitación, el valor de este conejo como factor, no es tan evidente.

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NOTES ON THE BIOLOGY OF LEISHMANIA TROPICA[1]

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The study of protozoa cultivated in artificial media has contributed in relatively small measure to our knowledge of their biology. Notwithstanding the numerous efforts made in this direction only a few protozoa have been succcessfully grown in artificial cultures in laboratories, and in many cases it has been necessary to make repeated inoculations, from one animal to another, in order to maintain certain protozoa alive.

In fact, successful culture of pathogenic protozoa has been made only within the last twenty years. Moreover, the failure of the first attempts at general use of cultures, and the results of these attempts, incorrectly interpreted, have been partially responsible for chilling the enthusiasm which accompanied the first fruitful investigations in this field of proto-zoology.

Alongside the brilliant results of Novy and McNeal, who first successfully cultivated trypanasoma in artificial media, the partially successful results of Bass with hematozoa, and of Ziemann with piroplasma, we must place the first fruitless attempts at amoeba culture, which led countless investigators to false interpretations, and also the failures in attempts to cultivate many other protozoa.

Leishmania cultivation, to which we have given our particular attention has been effected by many investigators, and all, or at least the majority, now prefer the medium advised by Nicolle and generallly identified by his name, for the initial cultures and for the maintenance of the parasites, since in addition to simplicity of preparation, it presents the advantage of allowing Leishmania to develop abundantly and to maintain vitality during many weeks.

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[1] Translated from the Portuguese by the Division of Medical Education, Rockefeller Foundation.

Going back to the first attempts to cultivate Leishmania in an artificial medium, we find that Rogers was the first to secure cultures of this genus of trypanosome, by cultivating Leishmania donovani. Rogers' cultures were obtained in blood suspended in a physiological salt solution, to which 5 to 10 per cent sodium citrate had been added.

Rogers' attempts, crowned with success in cases in which the culture was made in a liquid medium, did not yield the same result when he attempted to make a culture in a solid medium. In view of the slight development of the parasite, he attempted, with success, to obtain better development of this protozoa by slightly acidulating the medium with citric acid.

Later Nicolle cultivated another species of this genus, the Leishmania tropica, not only in a liquid medium as Rogers had done with Leishmania donovani, but also in a solid medium, prepared according to the technique used by Novy and McNeal in the cultivation of the Trypanosome of birds.

Still later Nicolle simplified the medium somewhat, making it more suitable for the experimental study of Leishmania.

Nicolle's work was confirmed by Marsinowsky, by Brogrov, and by the present writer, in collaboration with Dr. Pedro Dias da Silva.

Our own work followed the studies of A. Lindenberg, who first identified the "Bauru ulcer" (of Sao Paulo, Brazil) with the ulcerous manifestations in the Orient produced by Leishmania tropica. We made many studies of this parasite in culture of material obtained from cases of Leishmania in the wards of the Sao Paulo Hospitals, and succeeded in verifying the capacity of this protozoa to develop not only, as has been supposed up to date, in the condensation water, but also in the solid portions of the medium, where it forms a clearly visible path, difficult, however, to distinguish from the colonies of bacteria, of penumococci, etc.

After Nicolle's medium, countless other media were proposed for cultivation of Leishmania, but none of them present actual advantages which would justify their systematic use.

Among the many culture media proposed, we should mention that recommended by Astrogildo Machado, which contains glucose and in which the protozoa develop most rapidly and abundantly.

The growth of the protozoa is facilitated by the presence in the medium of carbohydrates, which facilitate the development of the organisms since, as do bacteria, they attack the carbohydrates first, before acting on the proteins.

However, for reasons not yet fully evident, they do not act on carbohydrates when placed in non-albuminos media.

We have for several years been making investigations upon the problem of culture media which are favorable to the development of Leishmania. Before going into the details of these investigations, we desire to discuss briefly the technique of cultivation.

Leishmania being very slow in development, compared with bacteria and fungi, it is natural that we should desire to obtain the material for cultivation from a zone of tissue where it still exists unassociated with any other organism or, if this is impossible, from only slightly contaminated regions. For this purpose it is always preferable, to withdraw material from a nodule before tissue necrosis has proceeded very far, or from a sore in process or cicatrization. All the customary aseptic precautions must be carried out in removing the bit of tissue.

When material is withdrawn from a sore in process of cicatrization it makes little difference whether or not the patient has already received rather extensive treatment, since Leishmania is one of the last organisms to leave the sore. It is possible at times, by removing the hard adherent scab of a healing lesion, to obtain a bleeding surface which is soft and completely free from microorganisms other than Leishmania.

By compressing the sore between the fingers the granulated surface may be scraped and transferred to various tubes of blood agar, the same being done with the blood which flows from the wound.

A few tubes will show Leishmania cultures contaminated with one or more colonies of bacteria or fungi, while others will have a pure culture of Leishmania.

The condensation water should never be allowed to flow over the medium, except immediately after the material has been inoculated.

Once the culture has been started, it may be maintained in good condition in the laboratory, if re-inoculations are made every four or six weeks.

It is interesting to note that fragments of granulation tissue containing Leishmania, when placed in the condensation water, give rise to cultures—a fact leading us to assume that in the region around the tissue there exist conditions of anaërobiosis, which would indicate that Leishmania is a facultative aërobe.

The same observation may be made when blood clots are placed on the medium, and small colonies are seen to appear in the clot.

However, in experiments which we undertook to prove the possibility of Leishmania developing under conditions of anaërobiosos the results were absolutely negative.

Using the well-known, and almost universally adopted, Nicolle medium, we investigated the requirements of Leishmania as to the percentages of the various ingredients of which this medium is composed.

We used in all our tests 2 per cent agar, varying only the percentages of blood and of salt.

When we varied the percentage of blood we found that Leishmania began to develop as soon as a few drops of blood were scattered over pure agar, in 0.5 per cent salt.

As the percentage of blood is increased the rapidity and intensity of Leishmania growth increased proportionately, until the blood has reached the proportion of 15 per cent, which represents the maximum point of growth. Hardly any variation is noted from this point up to 30 per cent of blood, from which percentage the medium becomes less effective and the results less consistent.

From the point of view of economy the use of a smaller percentage of blood is advantageous, and we regularly used 15 per cent blood in our media.

Investigating in turn the proper quantity of NaCl to be used, we arrived at the following results:

When the medium contains 0.1 per cent of salt initial growth is very scanty, and a large colony never develops. In the proportions of 0.2 per cent development is less scanty, but is still imperceptible on the solid part of the medium.

When the amount of salt is increased to 0.3 to 0.4 per cent the initial growth is remarkable and is better than in the ordinary medium. As early as three days after transplantation it may be noticed that growth of Leishmania in the media containing this proportion of NaCl is greater than in those with any other quantity of salt. This holds true for ten days longer, from which time on it is difficult to note any difference among the various cultures, up to those containing as much as 2 per cent salt.

We believe therefore, that for cultivation of Leishmania tropica the most suitable medium is one with the following formula:

Water	1 liter
Agar	20.0 grams
NaCl	3 to 4 grams

To 8.5 cc. of this melted agar, maintained at a temperature of 45 to 50°C. there should be added 1.5 cc. defibrinated rabbit blood.

Technique. In adding the defibrinated blood to the agar, it should be allowed to drop from a graduated sterilized pipette on to the wall of the tube, to avoid producing bubbles, and the mixture must be made on the same side of the tube, leaving the other quite clean to facilitate observation of the culture.

It is usually advised that the blood and agar should not be mixed at a temperature higher than 56°C., the principal motive for this precaution being the danger of coagulating the blood albumins. This advise is, however, misleading since thep point of main interest is to see that the blood be used to the best advantage.

In winter when the agar cools rapidly we see no objection to taking the temperature of 56°C. as a maximum, but in our climate particularly where this temperature is reduced very slowly, this requirement is very inadvisable, since it gives time for the blood to deposit itself in the lower part of the tube and the medium becomes poorer in blood than the experimenter assumes it to be.

A medium prepared with the proportion of blood which we advise, if added to agar at the temperature of 56°C. forms on the upper part of the agar a layer in which the quantity of blood is very scanty, with a lower layer which is correspondingly over-rich in blood elements.

Fungi are the frequent contaminating organisms of the culture media in our climate, and it seem needless to us, in working with Leishmania culture media, to place the tube in the incubator for forty-eight hours, all the more so since our own impression is that this procedure partly destroys the efficacy of the media.

In concluding this section of our investigations we studied the reaction of the medium on the growth of Leishmania, and were able to confirm Rogers' statement that the addition of one or two drops of citric acid to the medium improves conditions for development of Leishmania.

In preparing the medium we added to the melted agar whatever quantity of acid or alkali we desired, followed by the rabbit blood.

In the following table the technique employed will be shown more clearly as well as the results of the investigations:

TABLE 1

QUANTITY 2 PER CENT GELOSE IN 0.5 PER CENT OF NaCl	QUANTITY DECINORMAL HCl	RABBIT BLOOD	DAYS					
			2	5	9	11	20	25
cc.	*cc.*	*cc.*						
8.4	0.1	1.5	+	+	+	++	++	+
8.3	0.2	1.5	+	+	+	++	++	+
8.2	0.3	1.5	+	+	+	++	++	+
8.0	0.5	1.5	+	+	+	++	++	+
7.7	0.8	1.5	++	++	++	++	++	+
7.5	1.0	1.5	+	±	±	—	—	—
7.3	1.2	1.5	+	Rather slow			—	—
7.0	1.5	1.5	±	+	—+	—	—	—
6.5	2.0	1.5	—	—	—	—	—	—
6.0	2.5	1.5	—	—	—	—	—	—
8.5		1.5	+	+	+	++	++	+

When 0.1 cc. of N/10 solution HCl or even up to 0.5 cc. of this solution is added to a total amount of 10 cc. of the medium, the acid has no effect whatever on the growth of the protozoa during the first two days; 0.8 cc. of the HCl solution appears to facilitate development, while 1.0 cc. to 1.5 cc. of acid, though not fatal to the Leishmania prevents their growth, and no active Leishmania are found in such media after the ninth day. When 2 cc. or more of the acid is added the Leishmania are destroyed in two days.

From the ninth day onward no further difference can be noted in the growth of Leishmania in the tubes containing up to 0.8 cc. of the N/10 solution of HCl, all of these having attained their maximum growth. In tubes containing more than 0.8 cc. N/10 solution, no live Leishmania are found after the ninth day.

The results of these investigations as to acidity of medium are entirely relative, since besides the blood, which inevitably alters the absolute acidity of the medium, there are other factors working in the same direction.

In media of this nature it is probably impossible to determine the concentration of the hydrogen ions by any of the colorimetric methods.

Let us now turn to our experiments on the effect of alkalinity on the development of Leishmania. We used the same technique as in the previous experiment with slight modifications in the tubes having a greater degree of alkalinity. We obtained the following results:

The cultures observed on the 6th day after inoculation show moderate growth in the tubes containing 0.1, 0.2, and 0.3 cc. of deci-normal solution of NaOH; in the tubes containing 0.5 and 0.8 cc. development is more advanced, and in the tube containing 1 cc. growth is moderate, becoming even less when alkalinity has increased by adding 1.5 cc. of N/10 NaOH.

At the same time that we made these experiments with decinormal soda solution we made observations with normal soda solution and found that when 0.1 and 0.2 cc. of this solution (N/1 NaOH) is added to the medium the Leishmania cultures continue their development, though not to any great extent, and that in tubes containing 0.3 cc. normal soda or more, Leishmania do not show any signs of activity.

Twelve days after transplantation growth of Leishmania was identical in all the tubes containing up to 1 cc. of deci-normal solution of NaOH, development being moderate in tubes containing a higher percentage.

This result surprised us, since the alkalinity resulting from the soda is added to the natural alkalinity of the rabbit blood, which must, of course, increase the total alkalinity of the medium.

In the acid and in the alkaline medium it is easy to predict in which tubes Leishmania will not grow, since when the quantity of acid or alkali is small no alteration is observed in the characteristics of the medium, while as the quantity of acid or alkali increases its color turns from a bright to a dark crimson.

In the tubes in which this change of color is very pronounced no Leishmania will grow.

We see from these studies that the range of degrees of acidity or alkalinity within which development of Leishmania can take place is relatively great, and that slightly acid or alkaline media tend to shorten the latent period.

Throughout the course of the work, since we had to keep six races of Leishmania in our laboratories the great quantity of tubes of Nicolle medium which we required gave us a good deal of trouble. We, therefore, attempted to determine more accurately the exact requirements of Leishmania in respect to different media, by testing its hemoglobinophilic properties.

For the cultivation of Leishmania and Trypanosomes culture media containing blood (usually rabbit blood) have been used exclusively, and since up to date attempts to cultivate these protozoa in other media have not been successful, these parasites have been regarded as strictly hemoglobinophilic.

But when we make studies of trypanosomes at various stages of development we see that at certain periods they are able to live without the assistance of hemoglobin.

This applies to Leishmania especially when it is functioning as a parasite, exactly as occurs with the trypanosomes at times under similar conditions.

Thus if we consider each of the species individually we see that Leishmania, during its life as a parasite, lives within the cells of fixed tissue, or of the wandering leucocytes, this parasite being rarely found in leucocytes in the blood stream, or in the lymph spaces which drain the affected regions.

In saprophitic existence, or better *in vitro*, hemoglobin has been considered essential for the growth of Leishmania.

Trypanosomes, although they can live in the blood stream during a certain period of their evolution both in man and in animals, can also live and develop in the cerebro-spinal fluid, in the lymph spaces and within the fixed tissue cells.

Trypanosome cultures have been only in hemoglobinated media. In order to find out to what point the necessity for hemoglobin in the medium extends, we entered upon a series of experiments with serum of various animals, as free from hemoglobin as possible.

In pure serum of man, sheep, rabbit, horse, guinea-pig, dog, and goat, Leishmania will live only a few hours.

In coagulated serum of man, horse and rabbit, prepared according to Loeffler's technique, using a physiological solution instead of meat-broth, Leishmania die in one or two days; in goat serum they remain alive for a longer time. We, therefore, limited our investigations to media prepared with goat serum.

To a determined quantity of goat serum *plus* physiological salt solution we added a deci-normal solution of HCl in quantities from 0.1 cc. up, as in the previous experiment.

The total amount of medium in each tube was 10 cc. After the serum had coagulated, about 1 cc. of a 10 per cent solution of Merck's dry pepsin in physiological solution was added to the surface of the medium.

Although growth of Leishmania was not very active, we noted that there was multiplication of the parasites in all the tubes, even in those containing as much as 1 cc. of HCl, N/10 solution.

This growth is much more apparent in tubes containing more than 0.5 cc. of deci-normal acid.

At about the seventh day after inoculation abundant Crithidia may be observed either isolated or in rosette formation, and moving about rapidly in the condensation water.

Even in the solid portion of medium countless well formed colonies are to be observed, but these are smaller and flatter than those observed in the medium containing blood.

Subcultures in media prepared in exactly the same manner did not yield the results which we expected. At the end of 4 or 5 days after transplantation the Leishmania lost their motility, notwithstanding the abundance of the *inoculum.*

The pepsin solution was sterilized by filtration, and its sterility was verified before use. The coagulated serum did not present signs of digestion in the exposed portions, but in the portions covered by the pepsin the albumin appeared slightly transparent, and at the end of a few days the water appeared dense or viscous.

When we substituted trypsin (1 per cent solution) for pepsin, and soda for the acid, we observed no growth whatever of Leishmania, which succumbed rapidly in this medium.

Even with minimum proportions of trypsin, keeping the medium at the room temperature (the practice in all our experiments) the medium presented evident signs of trypsic digestion.

There remains no doubt in our mind as to the ability of Leishmania to develop in media which do not contain hemoglobin, in view of the numerous cultures which we obtained in coagulated serum. We were unable, however, to explain why growth ceased in the second tube.

In our experiments, our principal purpose was the study of the physiology of this species of protozoa, and the results showed us that the requirements of Leishmania are not so rigid as has previously been believed.

CONCLUSIONS

1. We did not succeed in obtaining positive results in experiments which we undertook with the purpose of determining if Leishmania can grow under conditions of anaërobiosis.

2. In our examination of the various ingredients of Nicolle medium necessary for proper growth of Leishmania we observed:

a. That rapidity and intensity of the cultural growth increases in proportion to increase in the quantity of blood up to 15 per cent, which is the point of maximum growth; from this proportion up to 30 per cent of blood there is no appreciable variation, and a proportion of 30 per cent or more is useless and even harmful.

b. That the addition of 0.3 to 0.4 NaCl favors initial development to a remarkable extent, and gives better results than the usual medium.

c. That the best formula for a culture medium is, in our opinion, the following:

Water	1 liter
Agar	20 grams
NaCl	3 to 4 grams

d. That it is inadvisable to effect the mixture of blood and gelose at 56°C.

e. That the addition of 0.8 cc. of deci-normal HCl solution seems to favor development of the porosity when the quantity of N/10 HCl reaches 1 cc. or more, these proportions being harmful.

f. That both in acid and in alkaline media it is easy to determine in which tubes Leishmania will not grow, since the excess of acid or of alkali makes the bright crimson color change to a dark shade, and in media in which this change is the most pronounced Leishmania will not multiply.

g. That the range of acidity or alkalinity which allows development of Leishmania is relatively great, while slightly acid or alkaline media tend to shorten the period of latency.

3. We are convinced that Leishmania will develop in media which do not contain hemoglobin.

a. In pure blood serum of man, sheep, rabbit, dog, and goat Leishmania will live only a few hours.

b. In the same serum prepared according to Loeffler's process with physiological solution instead of meat broth, the parasites die in one to two days, except in goat serum in which they remain alive for quite a long time.

CONCLUSIONES

1. Despúes de llevar a cabo varios experimentos con el propósito de determinar si los Leishmania pueden ser cultivados

bajo condiciones anaeróbicas, no pudimos obtener resultados positivos.

2. Al someter a exámen los ingredientes del medio de Nicolle, necesario para el cultivo de Leishmanias, observamos:—

a. Que la rapidez e intensidad del cultivo aumenta en proporción con el aumento de sangre hasta un 15 por ciento que parece ser el punto de desarrollo óptimo; desde esa proporción hasta un 30 por ciento no existe variación perceptible; una proporción de 30 por ciento o mayor es inútil y hasta perjudicial.

b. La adición de 0.3 a 0.4 de cloruro de sodio favorece en gran manera el desarrollo inicial y dá mejores resultados que el medio corriente.

c. Es nuestra opinión que la mejor fórmula para un medio de cultivo es la siguiente:

Agua	1 litro
Agar	20 grams
Clor. de sodio	3 a 4 grams

d. No es prudente el mezclar sangre y gelosa a 56°C.

e. Es perjudicial la adición de 0.8 cc. de solución deci-normal de HCl pues favorece la formación de porosidad cuando la cantidad llega a 1 cc. o mas de aquella.

f. Que en ambos medios, ácidos o alkalinos, es fácil determinar en cuales de ellos se están desarrollando los Leishmania pues el exceso de ácido o alkalino afecta el brillante carmesí cambiándolo en un tinte oscuro. En donde este cambio sea pronunciado los organismos no crecerán.

g. Que hay grados de acidez o alkalinidad amplios que permiten el desarrollo de los Leishmania mientras que medios ligeramente ácidos o alkalinos tienden a disminuir el estado latente.

3. Estamos convencidos de que los Leishmania pueden crecer en medios que no contengan hemoglobina.

a. En suero de sangre puro humano, y de ovejas, conejos, perros y cabras, los Leishmania viven solamente pocas horas.

4. En esos mismos sueros preparados de acuerdo con el proceso de Loeffler, con solución salina fisiológica en vez de caldo, los parásitos se mueren de uno a dos dias, excepto en el suero de cabra en donde viven por largo tiempo.

THE PRESENCE OF YEAST-LIKE BODIES IN THE BLOOD OF HUMAN BEINGS

A PRELIMINARY REPORT

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Yeasts and yeast-like bodies have long been associated with a number of diseases of human beings. The *Oidium albicans* has been recognized as the cause of thrush, the *Cryptococcus gilchristi* as the cause of blastomycosis, and in a number of local conditions yeasts or yeast-like organisms of the *Fungi Imperfecti* have been demonstrated. Ashford (1) has claimed that a yeast-like organism, which he has called the *Monilia psilosis*, and which has also been given the name *Parasaccharomyces Ashfordii* (2), was the causative agent of sprue. This statement has been questioned by others who have studied this disease (3) so that at the present time the relationship between the *Parasaccharomyces Ashfordii* and sprue remains somewhat uncertain. Studies have also been carried out by Anderson (2) and Hannibal and Boyd (3) concerning the occurrence of yeasts in human feces—in the course of which it has been demonstrated that both yeasts and yeast-like organisms occur in the stools of normal individuals. In a number of conditions thus, yeasts or related organisms have been more or less definitely associated with disease; however, most of these conditions due to infections by these organisms have either been of rather infrequent occurrence or most prevalent in regions outside of the United States.[1]

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[1] While we realize that sprue is probably more widely prevalent in the United States than is generally recognized (Wood: Amer. Jour. Med. Sci., 1915, **150**, 692; Jour. Amer. Med. Assoc., 1919, **73**, 165. Boyd: South. Med. Jour., 1920, **13**, 299) we accept for the time being its status as a disease of the tropical countries and of the southern part of the United States. However, probably even in the southern states sprue is not a common disease.

Certain observations which we have made recently suggest that yeasts or similar organisms are of wider importance than we have previously believed them to be, and further that we must attach to the conception of infection by the pathogenic yeasts certain qualities which were previously unrecognized. While possibly the number of cases which we report upon at the present time are rather few, we believe that even these few justify our statements above, and certainly they warrant and necessitate an extension of the work with these organisms in relation to disease.

Case 1. This case was one definitely diagnosed as sprue. Dr. R. M. W., who had been a missionary in Korea for eight or nine years, developed sprue some three years ago. He returned to this country during the year 1922 and was put in touch with us through the kindness of Dr. J. E. Cook. At the time that we saw him he complained of a sensation of distension, flatulency, indigestion and mild diarrhea; he slept poorly and was irritable. His general appearance was quite fair and he did not show emaciation. We obtained a specimen of stool and isolated a yeast-like organism, somewhat similar to the *Monilia psilosis* (*Parasacchromyces Ashfordii*). In addition, in a blood culture taken at the same time we obtained an organism identical with that found in the stool. Unfortunately we were not able to take a second blood culture in this case as the patient left the city. However, we hope to have the opportunity to examine this case at a future date. We believe that this demonstration of yeast-like organisms in the blood may possibly represent a definite contribution to the study and diagnosis of sprue.

Case 2. Because of the history and previous movements of this patient, we cannot definitely exclude the possibility of sprue. E. W., a native of Wisconsin, was during the war located at Camp MacArthur where he came into contact with soldiers, who has previously served in the tropics, and some of whom were suffering with diarrhea which may, of course, have been sprue. He did not have diarrhea while at Camp MacArthur, but after he was transferred to Camp Merritt he developed an attack of diarrhea of brief duration; however, after this, intermittently, this diarrheal condition lasted up to the time we saw him in the summer of 1922. His weight had fallen from 153 to 112 pounds. He did not show any stomatitis at any time and his stools were liquid and not sprue-like in character. Cultures of the feces and blood both showed the presence of a yeast-like organism. The organism has been isloated from the feces twice. We express our thanks to Dr. C. F. Wilhelmj for the privilege of examining and reporting this case.

Case 3. This case is apparently not sprue—certainly, the clinical evidence does not suggest this diganosis. B. R., a patient at the Jewish Hospital of St. Louis, whom we were able to examine through the kindness of the physicians of the Medical Service of that Hospital, was born in Hungary, came to this country in 1903 and lived for a short time in New York before he moved to St. Louis. In 1921 he was operated for appendicitis. His convalescence was slow and he had considerable abdominal discomfort for many months afterwards. Finally in January, 1922, what was apparently an abscess in the abdominal wall was opened; the abscess cavity, from all appearances, communicated with a walled off peritoneal abscess. This has continued to discharge and failed to heal ever since. Meanwhile the patient suffered with intermittent and rather mild diarrhea, blood being present in the stool; at no time, however, was the diarrhea of serious moment. The patient has not had a stomatitis and the stools have not been the typical sprue-like stools. Yeast-like organisms have been found six times in the stool, twice in the discharge from the fistula and three times in the blood.

In these 3 cases then we find certain common features, a more or less persistent, chronic diarrhea and the demonstration of yeast-like bodies in the feces and the blood. Otherwise, one was a case diagnosed as sprue; at the time that we saw it, however, it was relatively inactive. Case 2 had never shown either the typical sprue-like stools, nor any evidence of infection in the mouth and had been considered as a case of persistent diarrhea possibly due to tuberculosis of the intestine. The third case also showed none of the characteristic evidences of sprue and was also considered as tuberculous, although no definite diagnosis was ever made.

We wish to make it clear that we do not believe that we are dealing with a clinical entity in these cases, but rather feel that in at least some chronic conditions, in which diarrhea appears as a symptom, we must probably consider yeast-like organisms as playing an active part in the disease.

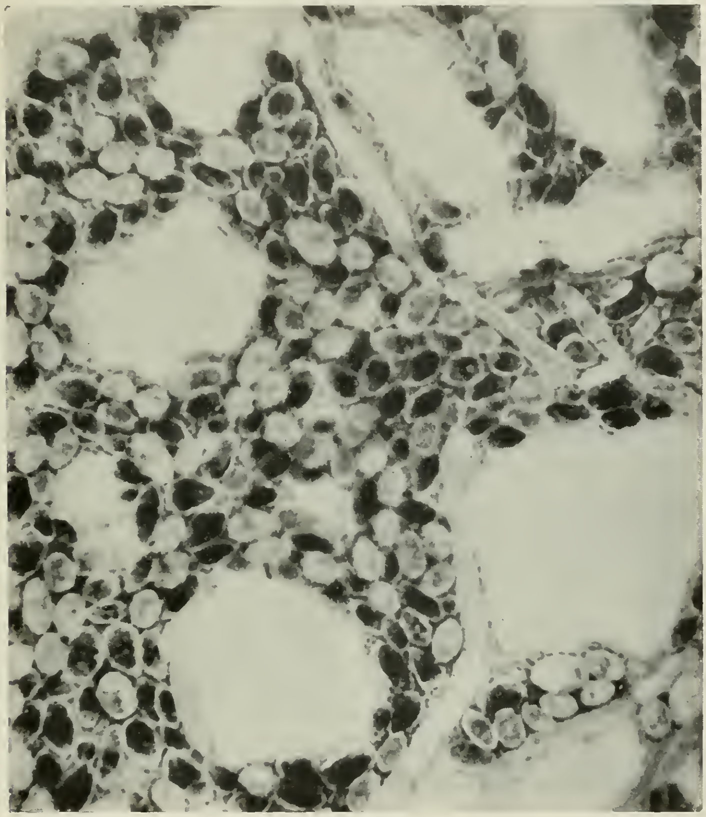
It was, of course, of importance to learn whether the organisms isolated from these 3 cases would also affect lower animals. We therefore fed (by the stomach tube) considerable quantities of cultures of these organisms to rabbits. Two rabbits were fed with the organisms of case 1. One rabbit died after three days showing a severe diarrhea, and the organisms were recovered from the intestine and also from the heart's blood at death. The second rabbit at first showed a diarrhea but survived for about four weeks, dying of an intercurrent infection; however, a blood culture taken from this rabbit on the third day after feeding was positive for the organism, and at thirty-six hours yeast-like organisms were present in the stool.

Three rabbits were fed with organisms from case 2. The yeast-like organisms were isolated from stool cultures of these animals, all of which showed intermittent diarrhea. One died after three weeks of an intercurrent infection and the organism was isolated from the intestine. Two were killed about three weeks after feeding. One showed no general lesions but the yeast-like organisms were found in the intestines and blood. The second showed generalized lesions and the organism was found in the intestine, lung and liver.

Three rabits were fed with organisms from the third case with the following results: One is still alive after about eight weeks; this animal showed diarrhea at first and the yeast-like organism was isolated from the feces. A second rabbit died eleven days after the feeding and the organism was found in the intestine and blood. The third rabbit died six days after feeding and again the organisms were recovered from the blood and intestine.

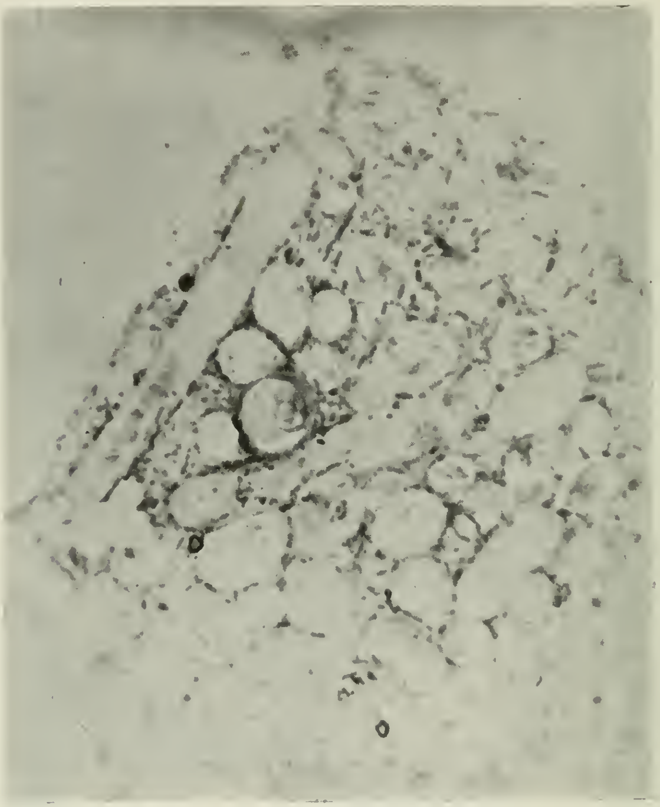
We omit here a detailed description of the autopsy findings in these animals, as the point which we principally desire to bring out is that it was possible to produce in laboratory animals by feeding the organisms essentially the same effects as were noted in humans. Previous investigators (4) who have studied the influence of yeast-like organisms in lower animals have reported death of the animals as a result of feeding and either subcutaneous, intralingual, intraperitoneal or intravenous injections. Usually, however, these investigators have not reported the recovery of the organisms from the blood in animals which were fed the organisms. Why they did not find the organisms in the blood we cannot suggest.

The morphology of the organisms isolated from the 3 cases was not identical. Those isolated in the first case (one presumably of sprue) showed oval yeast-like cells, varying markedly in size, some small and dark staining, others larger and paler; numerous elongated forms were also seen and these latter tended to be slightly granular. Nuclei were visible in these yeast-like cells. These cells tend to have flattened extremities, giving a rather



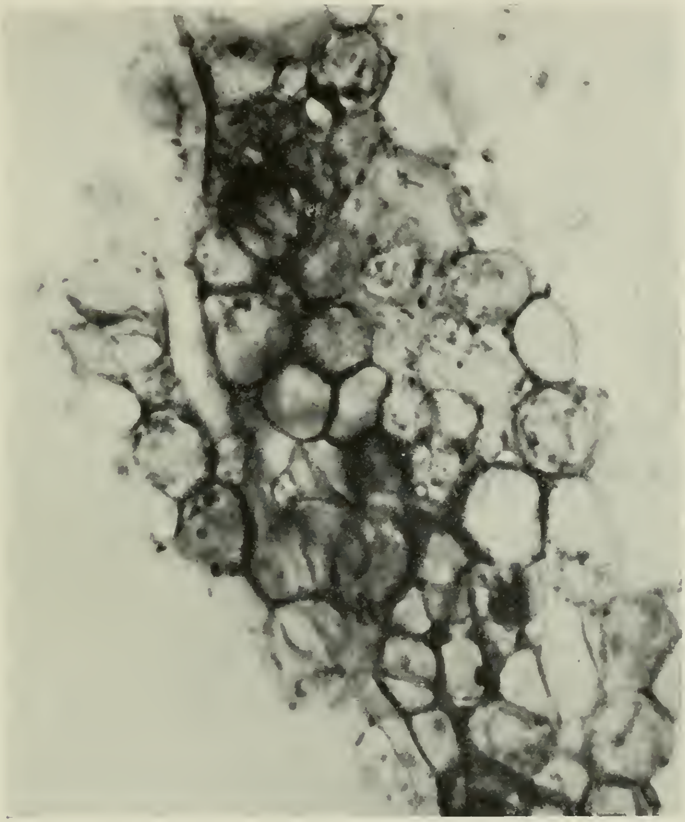
Case 1

square cut appearance to the ends. Mycelia are found and these show at times a profuse branching. This organism does not resemble exactly the *Parasaccharomyces Ashfordii,* a culture of which we obtained through the courtesy of Professors H. W. Anderson and F. W. Tanner, of the University of Illinois (fig. 4).



CASE 2

The organism found in the second case was more distinctly oval, usually stained rather poorly, but a few small darker staining cells were noted. Usually very few granules are seen in the yeast-like cells or in the elongated forms. There is the usual great variation in the size of the yeast-like cells. Mycelia are found and these show branching, but the mycelia stain rather diffusely and individual cells are not as readily distinguished as in the mycelia of cases 1 and 3. However, those mycelia appear to be septate. The organism from the third case is oval and very similar to that of case 1, excepting that the ends are more distinctly rounded and the organisms, especially the elongated forms, are more granular. The mycelia are usually short and show less branching than do those of case 1.



Case 3

It appears thus from the morphology of these yeast-like organisms that in all probability they are not identical; however, in view of the great variations in their size and outlines in any single culture, and the differences noted between young and older cultures, we prefer at the present time to make no attempt to classify them. Nor, in view of the differences in classification and nomenclature by medical workers and botanists, do we wish to make even a tentative classification until we shall have completed more extensive morphological, cultural and possibly serological studies.

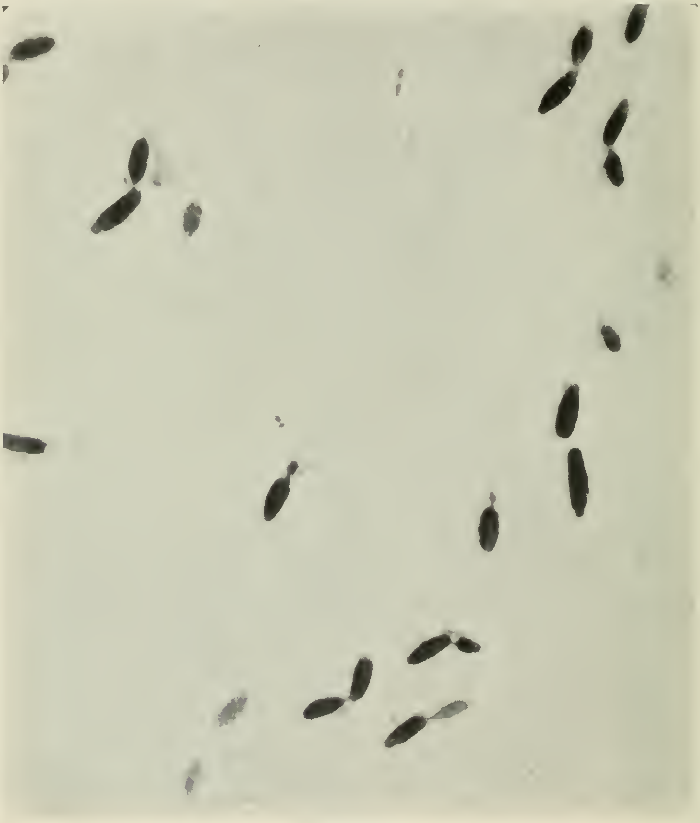


Fig. 4

The cultural methods used were as follows: For the cultures from the stool we used Sabouraud's test media—replacing the

French peptone by an American peptone (Difco)—(using 4 per cent of maltose C. P.). The fecal material was diluted or broken up in 0.85 per cent sodium chloride solution and plates were streaked from this suspension. For the blood cultures the same solid media and a similar fluid media containing only water, peptone and maltose were used. The blood taken with aseptic precautions was added in quantities varying from 1 to 5 cc. to either of the two media.

We found it advisable to wait from one to three weeks before examining the cultures as apparently it required a considerable time for the yeast-like organisms to grow in any quantities. At from one to three weeks the colonies appeared as white, glistening, raised and rather dense colonies, distinctly opaque and in the case of the fecal cultures quite different from the surrounding colonies of bacteria. After the yeast-like organisms were isolated, they grew readily and quite rapidly on the Sabouraud test media.

CONCLUSIONS

Dealing as we do with a small number of cases, we must of necessity be cautious not to go too far in generalization. The finding of yeast-like organisms in the blood of individuals suffering with chronic diarrhea is certainly of interest and further investigation may possibly prove it to be of quite considerable importance. The fact that these organisms fed to animals produced diarrhea and death, and that the organisms could usually be recovered from both the intestinal mucosa and the heart's blood at autopsy, appears to confirm their etiological relationship to the disease in human beings. The finding of the yeast-like organism in the one case of sprue certainly suggests that further studies must be made in this disease, and we are at present continuing these studies.

We wish in particular to insist that we are not reporting here on a clinical entity, but wish only to call attention to the occurrences of yeast-like organisms in the blood of human beings. Before we can discuss the full clinical significance of the presence of these organisms in the blood, a large number of cases must be studied both clinically and bacteriologically. We are now attempting to collect such a series of cases.

RESÚMEN

En tres casos de diarrea crónica obtuvimos, mediante cultivos, organismos pertenecientes al órden de las levaduras patógenas (*Saccharomyces*). Estos mismos organismos administrados a animales produjo en ellos diarrea y muerte y a la autopsia se recobraron de la mucosa intestinal y sangre del corazón. Esto parece confirmar su relación etiológica con la enfermedad en los humanos. El hecho de que uno de los casos era de esprú nos ha sugerido un nuevo campo de estudio que ya hemos comenzado.

Debemos hacer constar que no es nuestro objeto informar acerca de una entidad clínica, sino simplemente llamar la atencióm sobre la presencia de estos organismos en la sangre humana. Antes de poder considerar con amplitud la significación clínica de este hecho debemos antes estudiar un gran número de casos clínica y bacteriologicamente. Nuestras observaciones, sin embargo, demuestran que debemos conceder mayor importancia al concepto de infección por las levaduras patógenas.

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NOTICES

FIFTH CONGRESS OF THE FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE

At the fourth Congress of the Far Eastern Association of Tropical Medicine which was held at Weltevreden, Java, in August, 1921, a proposal was made on behalf of the Governments of the Straits Settlements and Federated Malay States by their delegate Dr. A. E. Horn, that the next Congress of the Association be held in Malaya.

The laws of the Association provide that the meetings shall be held every two years, and as the proposal was accepted, the Congress will meet in Malaya in 1923.

Dr. A. E. Horn was chosen President for the forthcoming session.

Drs. A. L. Hoops and R. Dowden were elected as Vice Presidents for the Straits Settlements and Federated Malay States, respectively, and Dr. Scharff as Honorary Secretary for Malaya.

On April 21, 1922, a meeting was held to elect committees for the organization of the Congress. Subsequently other meetings were held, and definite schemes were prepared for the management of Congress. It has been decided to hold the meetings from September 3 to 17. The first week of this period will be devoted to scientific discussions, and the following week to excursions to points of medical and sanitary interest throughout Malaya.

The Goverments of the Straits Settlements and Federated Malay States have recognised the important functions of this Association, and are contributing a considerable sum towards the expenses.

The object to this Association is to promote the science and art of tropical medicine in the Far East. For this purpose the Association endeavors to unite into one compact organization the medical profession of the Far East, for the growth and diffusion of medical knowledge; to promote friendly international intercourse among physicians; to elevate the standard of medical education; to enlighten and direct public opinion in regard to the problems of hygiene; to form habits which may conduce to the prevention of disease among the native population; and to present to the world the results of such scientific observations.

The membership of this Association consists of the members of all duly constituted, regularly organized medical societies within the field of this organization, and all medical officials in the civil, military, naval, or other organized services of Governments within their territories.

It is requested that those who wish to submit papers will communicate with the Honorary Secretary as soon as possible.

BOOK REVIEW

Infantile Cirrhosis of the Liver. By S. K. Mukherji. Published by the Indian Record Book Department, Calcutta.

This interesting little book of 95 pages, deals with a condition that is claimed to occur only in India and concerning which almost nothing is known. Infantile biliary cirrhosis is barely mentioned in a few text books of Tropical Medicine, yet according to the author's statistics it has been responsible for from 500 to 700 deaths annually in Calcutta alone.

The period of greatest susceptibility is from six to twenty-four months of age. Infantile cirrhosis is diagnosed by: (1) Absence of all known etiological factors; (2) history of infantile liver in other children of the mother; (3) age, six to twenty-four months; (4) insidious onset; (5) enlargement of liver followed by contraction; (6) jaundice; (7) fever for which no other cause can be found; (8) clayey stools; (9) persistent constipation.

Both liver and spleen are enlarged. The liver presents the typical appearance of hypertrophic biliary cirrhosis both in the gross and in sections. The disease almost always results in death, though in certain instances when the diet is changed in time, recovery has occurred.

The most interesting part of the book is the discussion concerning the etiology of this mysterious disease. Syphilis, malaria, kala azar, parasitic infestation, and all ordinary possible causes are excluded. The author concludes that the disease has its origin in dietetic errors. It is not found in children that are completely breast fed, so that the cause is not to be found in any deficiency in mother's milk. According to the author infantile cirrhosis is caused by overfeeding particularly with starchy foods.

There is a peculiar custom in Bengal of feeding the child with a spoon whenever it cries. They are very fond and proud of their babies and feed them at all possible occasions with no regard to the age of the child or the capacity of the stomach. The cow's milk used is frequently adulterated with starch and sugar. In addition to this these young children are often fed with considerable quantities of starchy foods like barley, sago, rice and sweet meats. While it is common for the same mother to lose several children from this disease just as is the case in infantile beriberi, the etiology is absolutely different. Infantile beriberi is due to a deficiency in the mother's milk and is seen in infants whose food is mother's milk. When infantile cirrhosis is seen in several children of the same mother, the mother's milk is either deficient in quantity or quality and the children get very little breast milk but instead anything and everything from cow's milk to rice and sweet meats.

If the author is correct in his deductions concerning the etiology of infantile cirrhosis, these observations may have some importance in explaining cirrhosis of the liver in adults. Practically nothing is known concerning the etiology of cirrhosis. It has long been believed that over indulgence in alcohol was one of the causes of atrophic cirrhosis. But it cannot be the only cause for it occurs in people that have been total abstainers. But alcohol and carbohydrates are metabolized in the liver. It may be that some cirrhosis in adults is due to excessive consumption of carbohydrates, thus throwing a burden on the cells that they cannot manage, with the result that they degenerate and are replaced by fibrous tissue.

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DENGUE FEVER

A CLINICAL REPORT OF THE GALVESTON EPIDEMIC OF 1922

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Received for publication February 16, 1923

About thirty thousand cases of dengue fever occurred in Galveston during the summer and fall of 1922, and the observations which this widespread epidemic afforded form the basis of this report. The experimental work has not been completed, and it will be grouped in a separate paper, along with the epidemiological considerations.

This city is situated on the eastern end of a low flat sand-bar and is not well drained. Water stands in gutters and under many houses during a rainy season, and swamps which are used as dumping grounds for trash encroach upon the western portion of the city. Mosquitoes breed rapidly on the island, and since no concerted effort is made to combat them, large numbers can be found during the long heated season. *Culex quinquefasciatus* (*fatigans*) and *Aedes aegypti* (*Linn.*) (*Stegomyia fasciata*) are always present in considerable numbers, during this season, and usually the *Aedes aegypti*, or yellow-fever mosquito, predominates, but occasionally the two are found in nearly equal numbers.

During the first week of June the city was swept by hordes of yellow-fever mosquitoes, and for many days they constituted a plague. Unscreened houses of the poorer classes situated near, or around the dumping grounds, swarmed with these mosquitoes. They gained entrance to practically all screened houses, invaded automobiles, and countless numbers were present in the grass of overgrown gutters and lawns.

During the second week many cases of fever appeared in the western part of the city, and I believe Dr. E. M. F. Stephens of the United States Public Health Service was the first to recognize these cases as dengue fever. He had seen the epidemic of 1918 which was reported by Levy (1), and was well qualified to direct attention to the beginning of the recent epidemic. About five hundred cases appeared in this district before the disease began to filter into the city, but during the third week of June the spread became rapid, and toward the end of July, 8000 cases had occurred, at which time a preliminary report was made (2). During August and September there was no abatement, but in October the epidemic rapidly diminished, and near the middle of November it abruptly ended. Since that time sporadic cases have appeared, but none has been reported during the first half of December, at which time this is written.

The figures are approximate for several reasons. Comparatively few cases were reported to the Health Officer, and in many cases a physician was not called. It is estimated that about 60 per cent of the population was attacked; this estimate is based upon personal counts, percentages in entire blocks, on certain streets, among large groups of employees, and in boarding houses and dormitories, and is conservative.

The literature of dengue is extensive and has been carefully written; it seems unnecessary to review it again. Ashburn and Craig (3) published a complete review in 1907. Cleland, Bradley and MacDonald (4) contributed an important chapter in 1916 on the Australian epidemic, and added further researches in 1919 (5). The Formosa epidemic was reported in 1917 (6) and Lane reported 100 cases in 1918 (7). Craig (8), discussed the etiology in 1920, a phase of the disease which will be considered in a later paper in conjunction with Asa C. Chandler of the Rice Institute. In 1922 Bassett Smith (11) gave a short review of recent work, with a bibliography.

CLINICAL CONSIDERATIONS

Five-hundred and sixty-five cases of dengue were studied by me, and I am indebted to many members of the profession here for their assistance, observations, and suggestions. Their work includes in this report approximately 3200 cases. A part of the observations were made in the wards and out patient department of the John Sealy Hospital.

The diagnosis is made on five principal observations:

1. Sudden onset with fever, and a chill or chilly sensations.
2. Aching pains which may be general, or localized in the head, and back or legs.
3. Suffused face and eyes.
4. Normal or low leucocyte count with a relative lymphocytosis.
5. Absence of jaundice and albuminuria.

In addition to these fairly constant symptoms, one usually finds:

1. Soreness of the eyeballs and skeletal muscles.
2. Prostration and loss of appetite.
3. Alteration of taste.

All of which should be confirmed by:

1. The appearance of a toxic rash.
2. Termination of fever within three to seven days.
3. The absence of any other disease to explain the symptoms.

These observations have been described many times in the literature, but there seems to be a confusion of the main issue. Epidemics vary in intensity, and special features may be present in one that are not seen in another. I think we may accept that as a fact. The disease is not diagnosed by its variations, but by the symptoms that are manifest in all. In a wide-spread epidemic almost any common sign or symptom may be observed, but many of them are in no way diagnostic. In the following paragraphs some of the principal clinical manifestations will be consecutively considered.

The incubation period in dengue fever has not been determined unless one may say that it lies within certain limits. It has been placed between five and nine and one-half days (4). In our experimental cases the shortest incubation period was four days, two hours, and the longest six days, ten hours. Sixty-two clinical cases could be recorded with reasonable accuracy. Some of these entered screened rooms in the hospitals or private homes one or two hours after their arrival in Galveston. Others left the city and spent their vacations where dengue has not occurred, and developed the disease. In these the incubation period varied between six and fourteen days. In uncontrolled cases the usual time appeared to be five to ten days.

A sudden onset has been reported by all observers. There were mild prodromal symptoms; dull headaches, a feeling of tire, and a gradually rising temperature, which seemed to occupy eight or nine hours, and that increased rather suddenly with the actual onset of the disease, at which time a chill or chilly sensations were felt, and the temperature abruptly rose.

The fever curve was extremely variable. Following the peak at onset, it frequently descended to normal within twenty-four hours or less. In the majority of cases a level between 101°F. and 102.5°F. was maintained for two to four days, but we saw the peak vary between 100°F. and 105.6°F., and the duration vary between one and twelve days. The "saddle back," or double curve was occasionally seen, but early in the epidemic it was found that the relapse usually did not occur if patients were confined to their beds for two or three days after the return to normal, and "termination of fever within three to seven days" seems to be the justifiable conclusion. There appears to be a fairly constant febrile periodicity of eight or nine hours throughout the course of the disease. If this observation be accurate and can be confirmed during subsequent epidemics, it will prove valuable in experimental work. This possibility was not discovered until my own attack of September 9, which is representative.

At 8:00 a.m. while stepping into my car, I noticed a stiff, uncomfortable sensation in the lower back. There were no other symptoms and no temperature. The usual daily routine was carried out, with lunch at one, followed by two hours in my office. A sensation of lassitude, frontal headache, and muscular stiffness ensued during the afternoon, my temperature rose hourly to 102°F., and at 7 p.m. I had a sharp chill of thirty minutes' duration; at 8:00 p.m. temperature of 104°F. Then the symptoms were intensified, and the temperature oscillated every eight or nine hours for four days with remarkable regularity. No drugs of any kind were taken, and an ice bag was not used.

During the remainder of the month my interest lagged, and the difficulty of getting hourly temperatures and careful observations of initial symptoms, limited the cases to 9. Seven of the charts are very like my own, and since I had nothing to do but keep my chart, I shall copy it (chart 1) along with those which vary in relation to temperature (charts 2 and 3). Bradley's chart (4) shows an onset occupying about ten hours. Three dissimilar charts are presented (charts 4, 5 and 6), and a composite chart to show variability (chart 7).

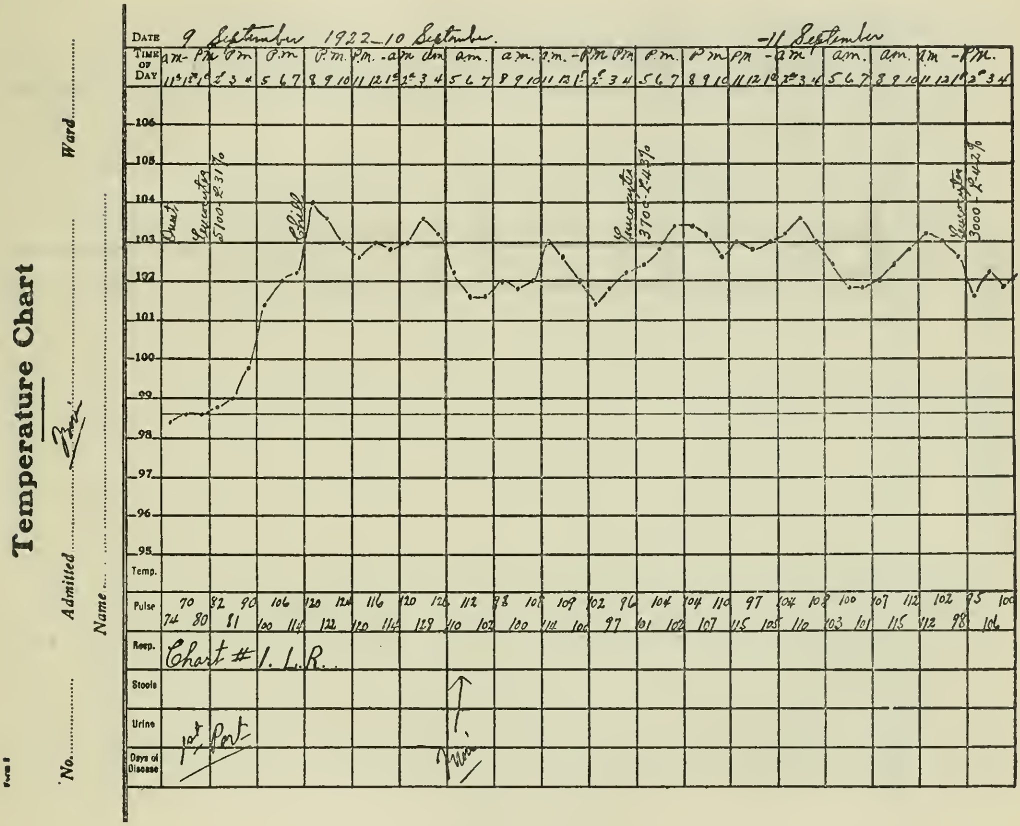

CHART 1.—1ST PART

The blood findings in dengue seem to be characteristic. Ashburn and Craig (3), and Stitt (9), carefully describe the picture, and we have steadily confirmed their reports. There is a normal or low leucocyte count with a relative lymphocytosis. I say normal because one may find, in such a large group of cases, focal infections which may prevent the occurrence of a leucopenia. The count usually varied between 7200 and 3100, the lowest being 1200 in a boy nine years of age. Neither did we find a constant curve in the lymphocytes as did Stitt, unless the cases be selected. Rather did we find a variation in the count from day to day, the highest count being 66 per cent.

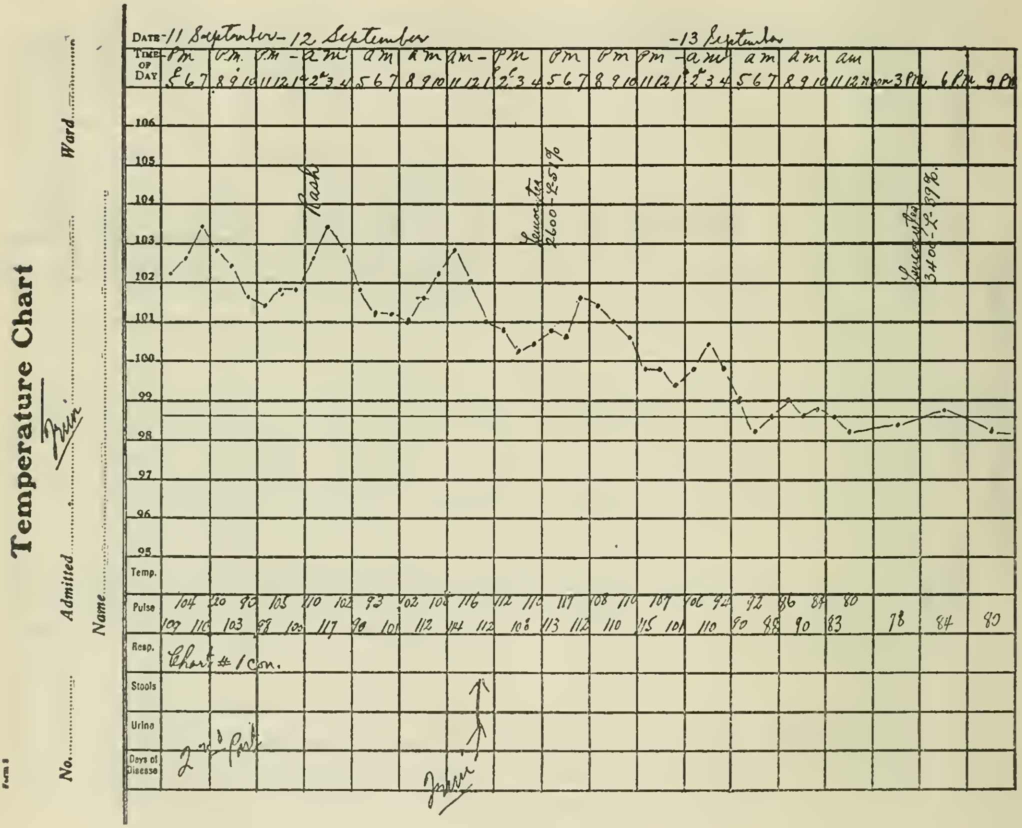

CHART 1.—2ND PART

The rash is toxic in character. That statement would allow it to assume many forms, and many forms have, in fact, been described. On the whole the rash observed in this epidemic consisted of low flat papules with a non-indurated base, which showed no tendency to coalesce. The papules were oftenest 0.5 cm. to 1 cm. apart, and appeared first on the flanks, lower abdomen, flexor surfaces, of the arms and back in the order named, but no rule was followed, and the rash sometimes appeared on the back or arms first. It frequently appeared toward the end of the three to five day period of fever, or along with a secondary rise, but with no regularity. I saw two patients with a well developed papular rash covering the entire body eight hours after the onset of noticeable symptoms, 116 with a definite rash

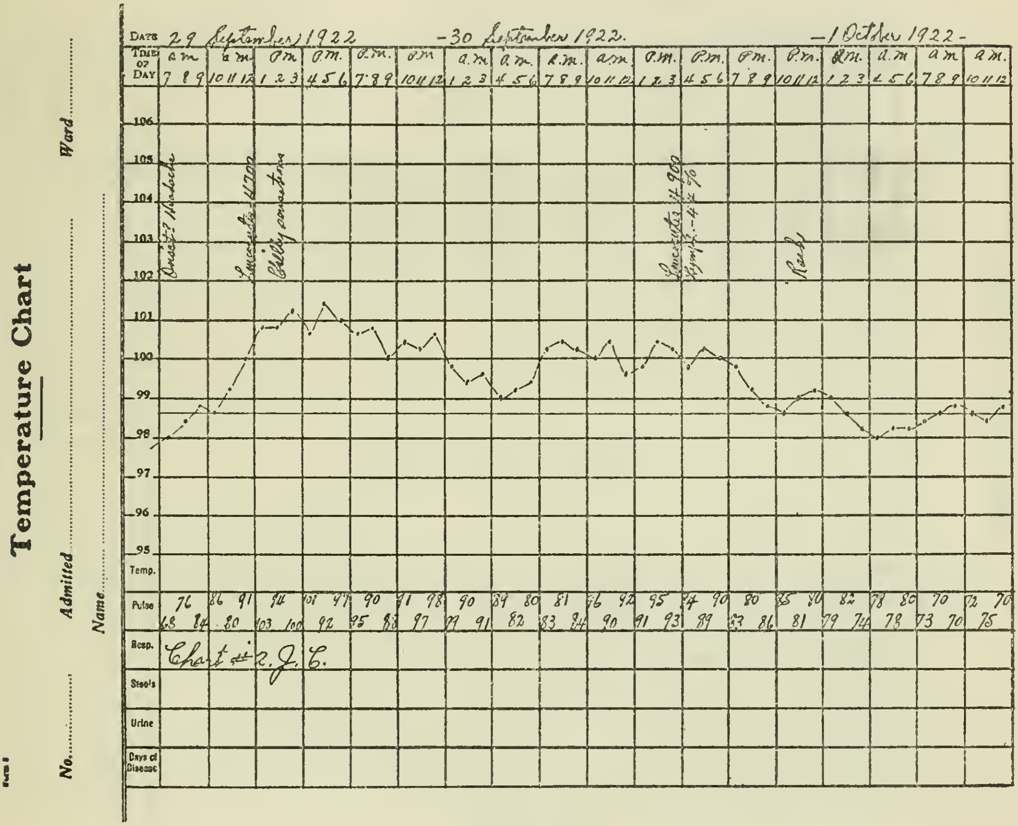

CHART 2

on one or more of the locations mentioned twenty-four hours after the onset, 193 on the second or third day, 76 on the fourth day, 105 after the temperature had returned to normal, or following a secondary rise, and in 73 a rash, other than the initial erythema, was not observed. I am inclined to believe that a rash is present in every case of dengue fever, although it may be of only a few hours' duration. Many patients were seen after dark during the height of the epidemic, and a diffuse rash could easily have been overlooked. Seventeen patients had an urticarial eruption with itching and sensitiveness of the skin; 42 presented capillary hemorrhages into the papules; 5 showed

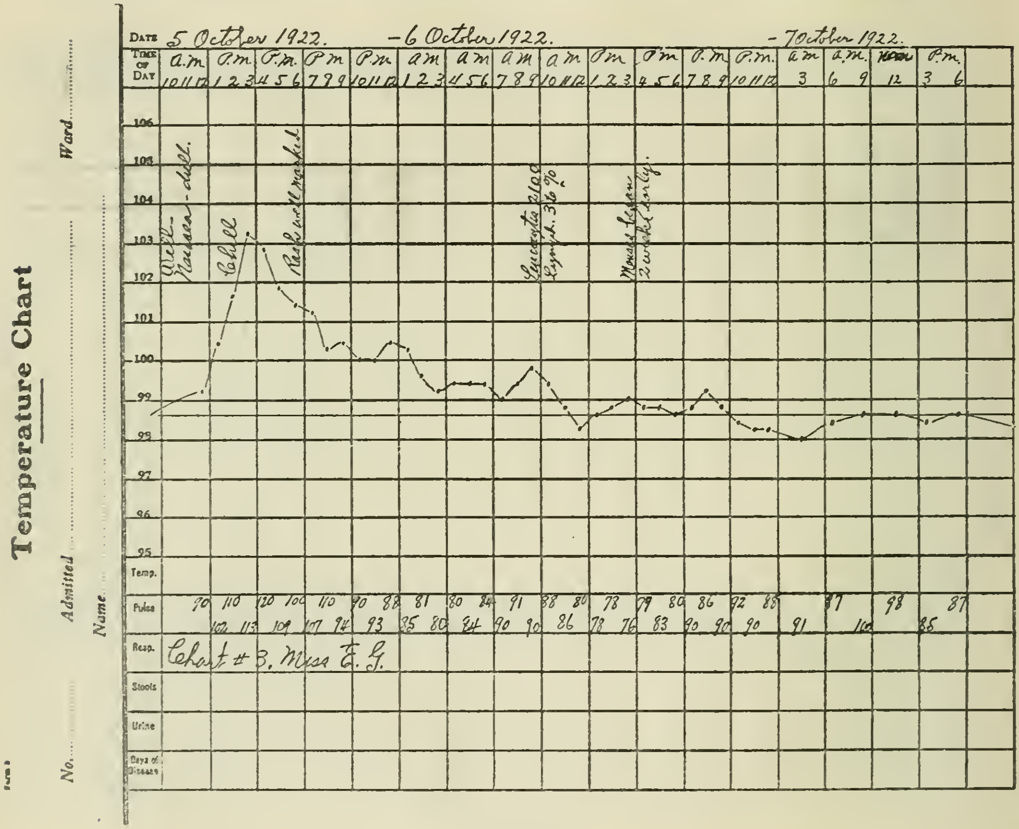

Chart 3

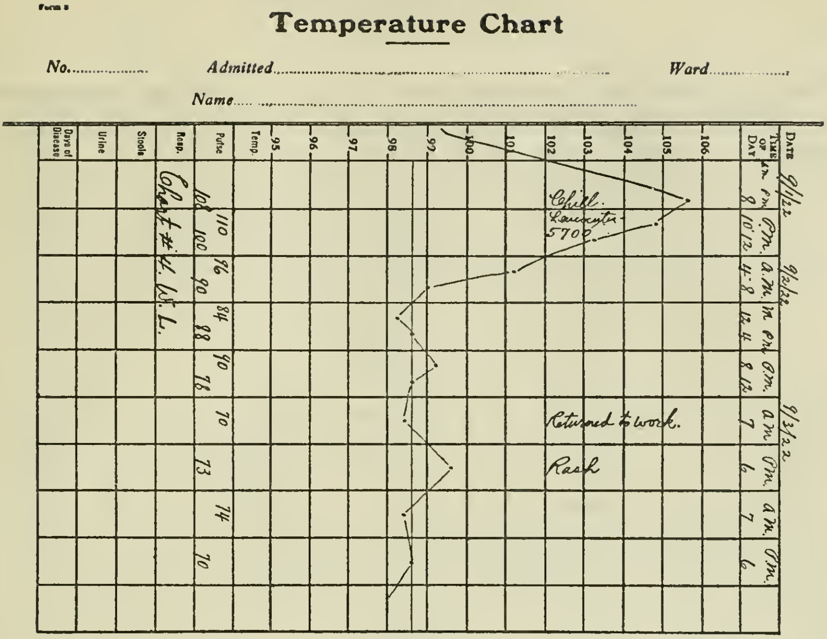

CHART 4

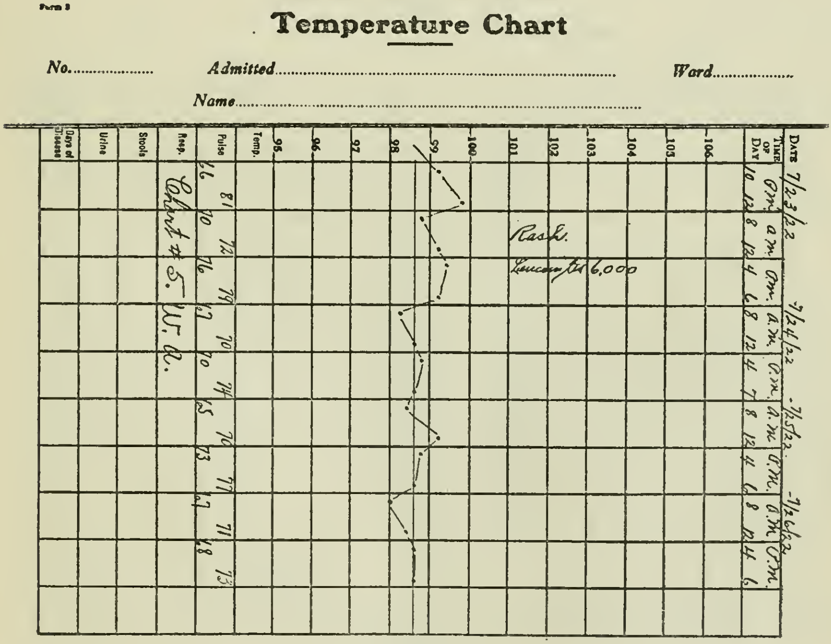

CHART 5

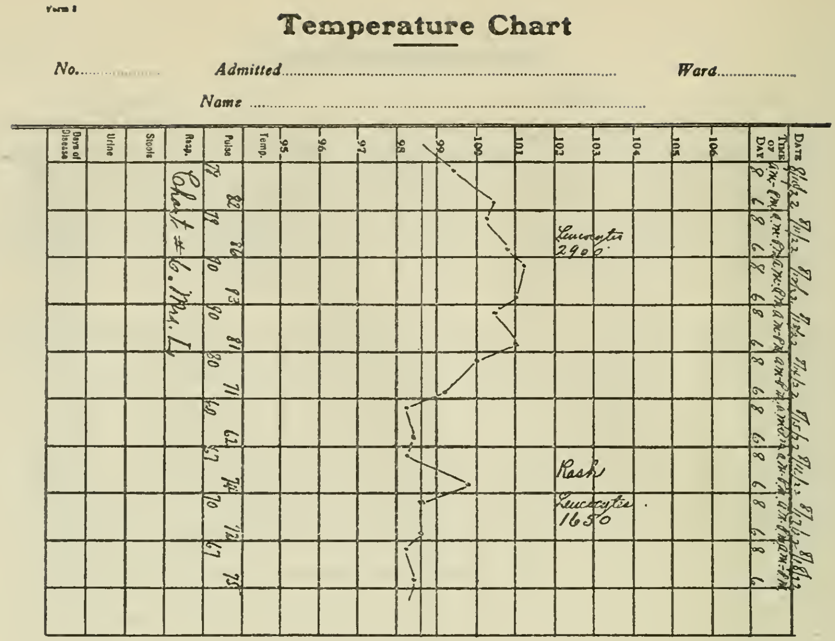

CHART 6

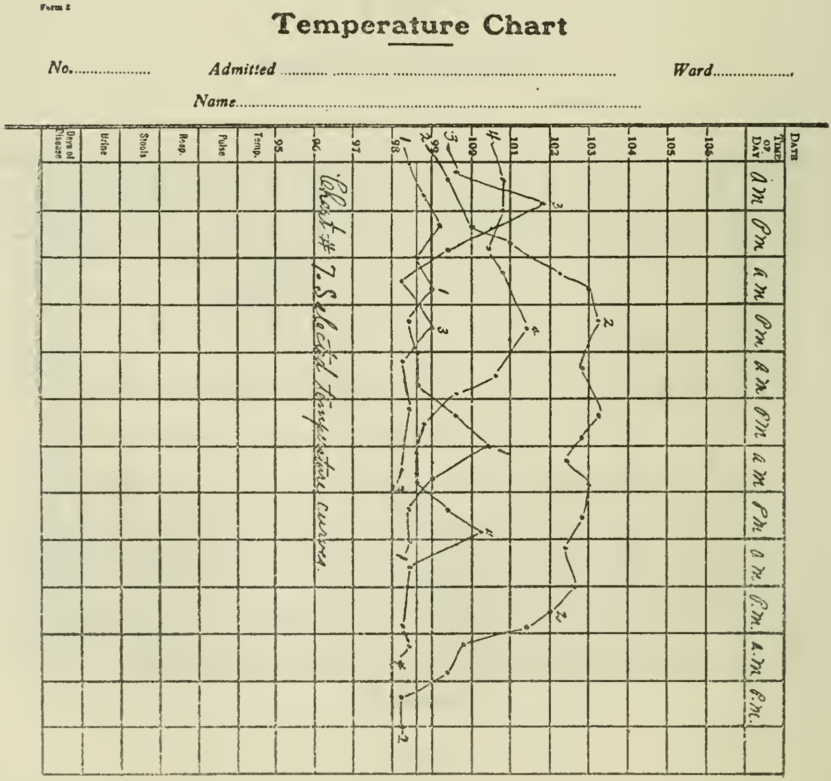

CHART 7

petechia from 3 mm. to 5 mm. in diameter; and small furuncles frequently appeared on the face and shoulders during convalescence, accompanied by itching of the palms and soles. Almost every one complained of pricking sensations over the body while the rash was present.

I have asked the dermatological consultant, Dr. E. D. Crutchfield, to describe the rash for me, and his report follows:

The skin manifestations in dengue fever

The recent epidemic of dengue fever in Galveston, and the opportunity to observe several hundred cases, has led to the following observations:

The most outstanding feature of the skin eruption in this disease is that it may be multiforme, or polymorphous. Nevertheless all the cases seen in my series may be grouped under five different heads. Certainly the eruption characteristics, as its sudden onset, its tendencies to be bilateral and symmetrical with some urticarial element present, suggests a great similarity to the erythema multiforme group.

The eruption seemed to show a predilection for the face, forehead, neck, upper chest, forearms, legs, palms and soles. In the cases observed in the maculo-papular group there was a predilection for the flexor surfaces rather than the extensor surfaces, as recorded by most observers. The diffuse urticarial infiltration in the palms and soles was a characteristic feature of quite a large per cent of the cases. In a considerable number of cases pruritis was intense, and in a lesser number a post eruptive itching of the most intense nature was seen, particularly when the eruption had involved the palms and soles.

Group 1. A diffuse erythema, most commonly seen on the face, upper trunk and extensor surfaces of the forearms. This may or may not be associated with considerable edema, and in a few cases typical giant urticaria has been coincident with the eruption. This erythema is the most constant of the skin manifestations seen in dengue. Occasionally the urticarial element is so pronounced as to form vesicles. This erythematous type of eruption is often seen as the so-called initial rash, and may be somewhat transient, or may be seen as a recurrent erythema rather than some other character of eruption, on about the fourth day of the disease.

Group 2. In the second group falls the eruption which is roseolar in character. Here dull reddish macules are present, sometimes becoming confluent. The macules are more sharply defined and more definitely circumscribed than the rash in measles.

Group 3. The third group comprises the papular type, which may be divided into the small papular when the lesions are small punctate papules, which in some cases may contain minute vesicles in the summit of the papule, or again may be small hemorrhagic papules.

Group 4. The other group of the papular eruption is usually maculopapular, of a reddish color, and often having an urticarial appearance. These lesions are sharply circumscribed and discrete, usually bilateral and symmetrically distributed.

Group 5. In the last group of cases the eruption may be said to be scarlitiforme in appearance. Here the eruption is composed of small punctate papules surrounded by an erythematous zone, the zones usually becoming confluent. This type of eruption occurs mostly in children and is not unlike the eruption seen in mild scarlet fever. As a whole the eruption in dengue fever may be said to resemble a toxic erythema.

Flushing of the face and injection of the eyes were seen in every case. This was a common observation. Usually the suffusion extended to the neck and chest, and in many to the trunk and limbs. The other signs of scarlet fever were not found.

Aching pains in the head were a constant symptom. They varied in intensity and were usually accompanied by a heaviness or dull sensation. In practically every case there was some stiffness and pain across the loins, and with these two locations as a starting point, one could elaborate many variations and conditions which have been described heretofore.

The urine may show traces of albumen during the disease, but this finding is not common, and since albumen is occasionally found in any group of patients, its absence, like the absence of jaundice, is the more important finding in dengue.

The tongue is usually coated, but so may it be in any acute fever, and other conditions. There is, however, an alteration of taste in almost every dengue patient. Salt tastes bitter, and flavors are differentiated with difficulty. The appetite is usually lost. Vomiting frequently occurs, and may become serious, as it did in seventeen cases. Constipation is the common finding.

The lymph nodes are frequently enlarged, especially the inguinal groups, and since this is not common to many fevers, the observation may aid in diagnosis. Bubonic plague should be thought of.

Hemorrhage is not a common manifestation, but it may occur from the mucous surface, and 75 per cent of women had menstrual disturbances. If they were stricken at the time, or during their menses, nearly all experienced a marked increase of flow which, in a few, became alarming. The others menstruated again, or too soon for their usual regular periods. Bleeding also occurred from the nose, ears and mouth. Goldsmith reported a fatal case in 1917. Dr. Edward Randall, Jr., described to me a very severe hemorrhage from the bowel during an attack of dengue which threatened his patient's life. Forty-seven gastric hemorrhages are known to have occurred; many were dark in color, and several constituted "black vomitus." These cases were interesting, but not misleading, since these patients had a rapid pulse and no jaundice.

The pulse followed the temperature fairly well, but it was found to lag to some extent, and in a few cases it was slow. The depressor effects of dengue are very prominent, and a rapid pulse would not be expected. I consider that the pulse is not diagnostic, but it deserves careful consideration when either typhoid fever or yellow fever are to be differentiated.

Jaundice was seen, and it undoubtedly occurs in the occasional case, but jaundice is seen in every epidemic, and during normal times. In 1897 it frequently appeared as a complication in the epidemic at Galveston, and usually indicated a malarial infection. The absence of jaundice is the diagnostic premise in dengue, and no yellow fever suspects were isolated this year.

Pregnancy was not disturbed. I have the opinion of men all over the State that abortions and miscarriages did not occur because of dengue. If the attack occurred toward the end of term, a pinkish discharge usually followed, and if at term or during the puerperium, hemorrhage was excessive, but no accidents occurred. I was unable to find an undoubted case of transmission to the fetus. That, however, seems to me to be possible, but, so far as I know, such an instance has not been reported.

Convalescence is slow and is attended by a sense of weakness, fatigue, and soreness of the muscles, which may last from one to six weeks. Boils may occur as mentioned. Chronic disease processes may make rapid progress, but there were no serious complications such as otitis media, gall bladder disease, acute appendicitis, or pyelitis, which usually attend epidemics of the exanthemata. Seven patients died while suffering with attacks of dengue, but each one had advanced disease processes (chronic nephritis, lymphatic leukemia, myocarditis, etc.) which would have destroyed their lives within a few weeks or months. No necropsy material could be obtained.

Immunity, at least partial, must be conferred since only 108 recurrences were discovered. The fact that re-infections were observed helps to support the conclusion, but the immunity is probably of short duration, and possibly does not exceed ninety days. During that time, however, the epidemic runs its course and disappears. The shortest interval between attacks was thirty-four days, and the longest eighty-seven days. Two patients had three well defined attacks. All recurrences were observed by the same men who saw the first attacks, and in no instance was the opinion of the patient accepted, although his diagnosis usually proved to be accurate. One's memory of an attack of dengue remains fresh.

Adults were attacked in larger numbers than either the very young or very old, but there is a greater proportion of adults than either of these classes. All ages were attacked in approximately equal proportions. Many babies had convulsions, and vomiting was an alarming symptom in many. A few required intravenous saline.

Treatment is simple and unsatisfactory. Salicylates, phenacetin, and codeine sulphate for the pain; sponges, ice caps, free intake of fluids, laxatives not purges, and rest in bed constitute the known treatment. The drugs named really have a doubtful place. Colloidal silver by mouth or intravenously is thought by some observers to be beneficial, but I am not prepared to discuss this method. The attack is self limiting, and those who are carefully nursed do as well as those who take sedatives. All should remain in bed for two or three days after the temperature has subsided.

The principal diseases with which dengue might be confused in the United States are:

Yellow fever which is transmitted by the same mosquito that carries dengue. In fact the belief is general that dengue is a mild form of yellow-fever, which, of course, is an erroneous idea. In the 1897 epidemic here Dr. John Guiteras isolated 8 suspects, and, following his lead, the city was quarantined. One of these patients had several remissions of typhoid fever following the initial attack for which he was isolated. Two others had malaria and promptly got well when quinine was administered. One had dengue with a gonococcus infection of the urethra, and another had dengue with chronic nephritis. Patients die with yellow-fever. The disease is characterized by jaundice, albuminuria, slow pulse and black vomit. As brought out above these signs are accidental in dengue and may be due to the effect of the disease on pathologic conditions already in existence.

Typhoid fever has a slow onset, rose spots, an enlarged spleen, bacilli in the blood, less prostration, a longer course, and a positive Widal or Marris. If in epidemic form the cases are limited to a water or milk supply.

Malaria shows some remission, if not intermission. The spleen is enlarged. A mild jaundice and labial herpes are common. Parasites can be found in the blood, and quinine cures the disease.

Scarlet fever has a brighter rash, prominent throat symptoms are almost constant, and there is a leucocytosis with a definite increase in the polynuclear cells. Desquamation occurs during convalescence. Children are usually affected.

Measles is accompanied by marked catarrhal symptoms. The rash occurs in crops and tends to coalesce. Koplik's spots are found. Desquamation follows the rash, and complications are common.

Influenza usually occurs during the winter months. It shows some respiratory symptoms, and they may be very prominent. The throat and fauces are red. If a rash occurs, it has a greater tendency to become petechial. The differential diagnosis presented no confusion in diagnosis when the Marines introduced dengue here in 1918.

Typhus fever shows a leucocytosis. Delirium is an early symptom. The temperature remains high, and a rash, which is petechial, occurs on the fifth or sixth day.

Trench fever usually runs a longer course, and the rash is less variable, although the pains are similar. The spleen is enlarged and there is a leucocytosis.

The onset of dengue was observed in women on the day of delivery, and on each day of the puerperium, but the low leucocyte count prevented unusual alarm. The same may be said of surgical operations. It is not a pleasant happening to find a temperature of 103°F. on the day following an uncomplicated appendectomy, but no incisions were re-opened, and the surgeons depended upon the symptoms enumerated and the blood count to make their differential diagnosis. Roseola infantum (10) presents some difficulty. It occurs in children under two years of age, and since I have seen none of these cases, Dr. Boyd Reading has written the following:

> During the past few years an increasing number of cases of so-called roseola infantum have been reported from various parts of the United States. In view of the fact that these cases bear a certain resemblance to dengue fever, which, during the past summer has been epidemic in the South, it becomes necessary to mention the points of difference between these two diseases.
>
> 1. Roseola infantum is prevalent during the colder months of the year. It has not occurred in epidemic form.
> 2. The rash always appears on the fourth day of the disease. It is a fine papular eruption most marked on the trunk. There is a tendency toward urticarial formation. Within twelve to twenty-four hours the eruption has disappeared, leaving a normal skin with no evidence of desquamation or itching.
> 3. Upon examination of the throat, there is a red papular eruption on the anterior pillars, with some redness of the tonsils and pharyngeal mucous membrane. An occasional ulcerated area may be present. According to J. H. Park, Jr., a diagnosis of roseola infantum can be strongly suspected by such findings in the throat before other evidences of the disease have become manifest.
> 4. There is no conjunctival inflammation, whereas in dengue, the velvety appearance of the lower eyelids is a very constant finding.
> 5. A leukopenia occurs in both diseases.

SUMMARY

1. The 1922 epidemic of dengue in Galveston followed a plague of yellow fever mosquitoes, and subsequently spread throughout the State.
2. The disease presents a few characteristic features and should not be confused with other clinical entities.
3. The rash may vary, but is distinctive.
4. Uterine hemorrhages are common in dengue, and hemorrhages from any mucous surface may occur.
5. Recurrences are seen, and partial immunity is of short duration, probably less than four months.
6. The incubation period varies between four and fourteen days.
7. Pregnancy is not disturbed.
8. No specific treatment is known.

I am indebted to Dr. Asa C. Chandler for his careful review of this paper.

SUMARIO

1. La epidemia de dengue en Gálveston en 1922 ocurrió seguida después de la invasión por una plaga de mosquitos de fiebre amarilla. Subsiguientemente aquella se extendio por todo el Estado.
2. La enfermedad presenta ciertas características y no debe confundirse con otras entidades clínicas.
3. La erupción varía pero es distintiva.
4. Las hemorragias uterinas en el dengue son comunes aunque hemorragias de cualquiera superficie mucosa pueden ocurrir.
5. Hay recidivas y la inmunidad parcial es de duración corta probablemente cosa de cuatro meses.
6. El período de incubación varía entre los cuatro y catorce días.
7. No afecta a la preñez.
8. No se conoce ningun tratamiento específico.

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THE TICKS OF PANAMA, THEIR HOSTS, AND THE DISEASES THEY TRANSMIT

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Ovid, New York

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The importance of ticks as transmitting agents of various diseases of man and animals continues to become more manifest as our knowledge of them increases. Although these annoying and dangerous parasites occur in practically all climatic zones they are much more numerous in the subtropical and tropical regions.

In Panama the ticks are extremely abundant as regard numbers and species, being found on nearly all varieties of domestic and wild animals, birds, snakes, lizards, toads, tortoises, etc., and also attacking man. The several tick-transmitted diseases of man, domestic animals and fowls, known to be present in Panama, cause these ectoparasites to be of far greater importance from an economic and health standpoint in this locality than they would be in other regions having a more temperate climate and freedom from tick-borne diseases.

In view of these facts a knowledge of the various species occurring in Panama is highly essential and studies were undertaken by the writer, during the several years while entomologist at the Board of Health Laboratory, Ancon, Canal Zone, to the end of determining the various species present, host relationship, relative importance, etc.

The following list contains only such species as were received at the laboratory from various sources and the identifications of which are quite certain. It does not include species obtained that are apparently new and undescribed or those of which a doubt exists regarding the identification.

ARGAS MINIATUS Koch

This is commonly known as the fowl tick or chicken tick. Chickens seem to be the preferred host but various other domestic fowls are also attacked. These ticks are very abundant throughout Panama, the majority of chicken houses and other places where fowls commonly roost in the cities of Panama and Colon, villages in the Canal Zone and native villages in the interior being usually infested with them. The larvae remain attached to the host for several days while becoming engorged, but the nymphs and adults feed at night and remain hidden in the cracks and crevices of the roosts, walls and floors during the day. This species is recognized as a serious parasite of poultry as it is the knowna gent of transmission of *Spironema gallinarum*, the organism causing spirochetosis of chickens. In cases of heavy infestation it also causes considerable damage by a general lowering of vitality as a result of the amount of blood taken form thechickens and the irritation caused by the bites. This disease has been diagnosed clinically in Panama, but as yet the organism has not been demonstrated.

ORNITHODOROS TALAJE Guerin

This is known as the human tick as man seems to be the selected host of the adult stages. It is very common in many of the houses in the native villages on the Isthmus. It somewhat follows the bedbug in its habits as it is a nocturnal feeder, engorges rapidly, and when not feeding secretes itself in the beds, walls and floors of the houses. The early stages of this species are also commonly found attached to rats in the two coast cities, Panama and Colon. The black rat, *Mus rattus* Linnaeus, and the brown rat, *Mus norvegicus* Erxleben, both act as hosts for the larvae and occasionally for the early stage nymphs. The brown rat seems to become the most heavily infested. One large male *M. norvegicus*, captured at the Panama city dump, with many larvae attached to it was placed alive in a tick breeding cage. During the following three days 58 fully engorged *O. talaje* larvae dropped from this rat. Twenty-one engorged larvae also dropped from a brown rat captured in a tenement house on West 12th Street, Panama, and 47 from a rat taken at Dock No. 4, Colon. However, despite the fact of this heavy infestation of rats in the cities of Panama and Colon with the early stages of this tick, none of the more mature stages have been reported in these cities and no mention ever made of their attacking man. This is the opposite to what occurs in the native villages where many of the houses are badly infested and the occupants nightly attacked by these parasites. More than 325 specimens were collected by the writer from a bamboo bed in a hut in the village of Arraijan. The larvae of this species seem to feed rapidly when on man but remain attached to rats for several days. Bates, Dunn and St. John (1) demonstrated by human experimentation that this species is a transmitting agent of relapsing fever in Panama. The Panama strain of relapsing fever spirochete transmitted by this tick has been recently proven by St. John and Bates (2) to be distinct from other species or strains of spirochetes causing relapsing fever in various parts of the world. A recent article by Darling (3) calls attention to the rat as a possible disseminator of relapsing fever in Panama. The evidence of this is based on the host relationship between the tick, man and rat and the susceptibility of man and rat to the disease and its transmission by the tick. This species of tick may therefore be considered as a very important one from a health standpoint.

DERMACENTOR NITENS Neumann

The tropical horse tick. This species nearly always adopts horses and mules for hosts although occasionally found on other animals. This tick is abundant in practically all parts of Panama wherever many horses are present. The inside of the ears seem to be the preferred place of attachment and in some instances animals are so heavily infested that the inner side of both ears may be a thick mass of attached ticks of this species, in all stages of development from unengorged larvae to engorged females. Observations (4) made on the bionomics of this tick in Panama show that the preoviposition period may be from five to seven days; the oviposition period from eight to sixteen days; 2401 eggs may be deposited by one female, and the shortest incubation period found to be twenty-five days. In January, 1913, Dr. S. T. Darling, (5) formerly director of the laboratory, detected in Panama a horse infected with *Piroplasma caballi*, this being the first record of this piroplasma in the New World. While it has not been definitely established that this tick is concerned in the transmission of equine piroplasmosis in Panama, all evidence is strongly indicative of it. In May, 1921, Dr. H. C. Clark, pathologist at the laboratory, visited Camp Gaillard to examine some Army horses that were sick. These horses had been used on a practice march to the village of Chorrera a short time previous and while there had been kept in a field that was sometimes used as a corral for native ponies. Of the 15 horses that were sick all were found to have piroplasmosis and all were also found to be infested with *D. nitens*.

RHIPICEPHALUS SANGUINEUS Latreille

The brown dog tick. Very common and practically all dogs in Panama, unless given very good care, are usually infested with this species. Between the toes and inside the ears seems to be the favorite places of attachment. This species is known to be the transmitter of *Piroplasma canis*, the organism causing prioplasmosis of dogs which is very common on the Isthmus. The screw worm fly, *Chrysomia macellaria* Fabricius, frequently deposits its eggs at the small lesions caused by the bites of this tick in the ears of the dogs, and which occasionally causes considerable damage to the animals.

IXODES RICINUS Linnaeus

The European castor bean tick. But one specimen, an unengorged female, of this species has been received, this having attached to Dr. H. C. Clark while hunting in the Boqueron River district in Panama. In other countries this tick attacks practically all varieties of domestic and wild animals and man. In Europe, where it is very common, it has been incriminated as a transmitting agent of bovine piroplasmosis. This specimen is quite probably the first one of this species collected in the American tropics.

MARGAROPUS ANNULATUS (Say)

The North American cattle tick. The transmitting agent of Texas cattle fever in the Southern United States. This tick has been found in Panama but it is not at all common. Considering the amount of livestock that has been brought from the States during the past eighteen years or since the American occupation of the Canal Zone, by the Panama Canal and individuals in Panama, it is strange that this tick is not found more frequently. The only specimens of this species secured by the writer in Panama were 3 males and 1 partly engorged female taken from a blooded bull that had been imported from the States about four months previously and had been kept in Panama City during the interval.

MARGAROPUS ANNULATUS AUSTRALIS (Fuller)

The Australian cattle tick. This is apparently a variety of the North American cattle tick and is very abundant throughout Panama, practically all cattle being infested. Although cattle are the principal hosts of this species, specimens are infrequently found on dogs. As this species transmits cattle fever it may be considered as the most important pest of cattle on the Isthmus. Although it had been suspected for several years that piroplasmosis was present among cattle in Panama, and several cases had been diagnosed from symptoms presented and pathological findings as apparently being cattle fever, no positive diagnosis was made until 1916 when Dr. H. C. Clark (6) succeeded in finding the *Babesia bigemina* in the brain capillaries of calves at autopsy. After this positive finding of the parasite in Panama investigations were carried out to determine the prevalence of the disease and the results obtained indicated that practically all of the native cattle were infected with a chronic form of the disease. The importance of this tick in connection with the transmission of the fever to imported non-immune cattle may be readily appreciated.

HAEMAPHYSALIS LEPORIS-PALUSTRIS Packard

The rabbit tick. This is the tick usually found infesting rabbits and hares, both domestic and wild, in many parts of North America. It occurs in Panama but is not at all common. Specimens were found on a rabbit in the laboratory stock that had arrived from the States about two months previously. Whether this rabbit was infested before leaving the States or whether it became infested after arrival in Panama through some of the wild animals which are quite frequently received at the laboratory is a question. Dr. S. T. Darling found specimens on an agouti, *Dasyprocta* sp., probably *Dasyprocta punctata isthmica* Alston, taken at Matachin, C. Z., in 1911, which would indicate that this species is present among the native wild animals.

HAEMAPHYSALIS KOCHI Aragao

Deer appear to be the principal hosts of this species. A number of specimens were collected at various times by Dr. H. C. Clark from white-tailed deer, *Odocoileus chiriquensis* Allen, near Frijoles on the Atlantic side of the continental divide.

AMBLYOMMA CAJENNENSE (Fabricius)

The Cayenne tick. This is the most important species of the genus *Amblyomma* present in Panama. It is the most abundant, most generally distributed and the most annoying to nearly all forms of animal life of any of the Panama ticks. It apparently has no preferred host but attacks man and all domestic and wild animals with equal freedom. Anyone who has traveled on the Panama trails to any extent, especially during the dry season, is quite certain to be familiar with all the stages of this species as they readily attach in large numbers. As yet this species has not been incriminated in the transmission of disease but from evidence presented in a case of myiasis in man which came to the attention of the writer (7) it is suspected that it may be concerned in the disposal of the eggs of *Dermatobia hominis* Gmelin.

AMBLYOMMA DARLINGI Nuttall

This species was first taken by Dr. S. T. Darling, in whose honor it was named, from deer and a black vulture, *Catharista urubu* Vieill. It closely resembles *A. cajennense* but differs in minor morphological details and ornamentation of both sexes. It is quite common and like *cajennense* has a variety of hosts, having been found on many domestic and wild animals in the Canal Zone, and has been taken from dog and wild turkey, *Crax panamensis*, in the Boqueron River region by Dr. H. C. Clark. While it probably attacks man no records of its doing so have as yet been received.

AMBLYOMMA AMERICANUM (Linnaeus)

The lone star tick. Specimens of this tick were taken by Dr. S. T. Darling, in 1914, from dog and hog on the island of San Miguel, one of the Pearl Islands group in Panama bay. While it is quite likely present on the mainland no record of its occurrence has been received. In the United States this species has been found on a variety of hosts, including man, animals and birds.

AMBLYOMMA DISSIMILE Koch

The iguana tick. Prevalent on many cold blooded animals, usually being found in abundance on the large iguana, *Iguana tuberculata*, the large toad commonly known as bullfrog, *Bufo marinus*, and many species of snakes, the large boa, *Boa imperator*, and the rainbow boa, *Epicrates cenchria*, nearly always being heavily infested. While making a survey of the reptilian fauna on the Isthmus the writer (8) found that more than 60 per cent of the snakes, 72 per cent of the toads and 84 per cent of the iguanas secured were infested with this tick. The replete females of this species sometimes become quite large; one specimen observed measuring 25 mm. in length, 15.5 mm. in width, 9 mm. in height and weighing 2.4 grams. This female oviposited over a period of thirty-five days; the total number of eggs deposited being 9254.

AMBLYOMMA LONGIROSTRE Koch

Taken from Rothschild's porcupine, *Coendou rothschildi* Thomas. Two specimens of this porcupine were examined and both were found to have many of these ticks attached. The deep penetration of the mouth parts of this species seems to cause considerable inflammation, resulting in a very noticeable lesion being present at the site of attachment of each tick. The spiny armament of the porcupine while serving as a defense against attacks of other animals apparently renders it helpless against attacks of ticks as the spines prevent the animal from biting or scratching them loose.

AMBLYOMMA NODOSUM Neumann

Adults of this species are commonly found on the three-toed anteater, *Tamanduas tetradactyla chiriquensis* Allen. A number of these animals were examined and practically all were found to be infested with this species of tick, the infestation being heavier during the dry seasons. On one specimen 36 males and 4 unengorged females were present, while a second one was found to have 41 males and 4 females (3 engorged and 1 unengorged) attached. The immature stages apparently seek other hosts as no larvae or nymphs of this species were found on anteaters. One engorged female of this species found in the laboratory beneath a cage containing a three-toed sloth, *Bradypus griseus griseus* (Gray), but in which an anteater had been confined several days previously (thus causing an uncertainty regarding the host from which it had dropped) measured 19 mm. in length, 13 mm. in width and 9 mm. in height.

AMBLYOMMA VARIUM Koch

Frequently found on the Hoffmann's two-toed sloth, *Choloepus hoffmanni* Peters. The thick pelage of this animal makes it practically impossible to find ticks on it except by feeling for them. One specimen of this species that dropped from a sloth in confinement was the largest of any tick observed on the Isthmus. It was an engorged female and measured 30 mm. in length, 24 mm. in width, 15 mm. in height and weighed 5.9 grams. After depositing 9759 eggs during a period of fourteen days this female died, apparently before the completion of deposition.

AMBLYOMMA HUMERALE Koch

Four specimens, 2 males and 2 females (1 engorged and 1 unengorged), of this species were taken from a tortoise found in the Boqueron River region by Dr. H. C. Clark. Both females were attached to the inner part of the edge of the carapace. The engorged specimen was 16 mm. in length, 12 mm. in width, 7 mm. in height and deposited 4589 eggs in a period of fourteen days. The tortoise was not preserved for identification but from the description received it seemed to be close to the South American tortoise, *Testudo tabulata.*

AMBLYOMMA PARVUM Aragao

Adults of this species were taken from a white-tailed deer captured on Ancon Hill. The larval and nymphal stages were found in abundance on cotton rats, *Sigmodon hispidus chiriquensis* Allen, captured in in the long grass near Balboa. A number of these rats were examined during February and March, 1921, and all were found infested with this species of tick. One rat captured in the grass south of the Balboa Commissary was found to have 2 nymphs and 22 larvae attached to its chin, being spread over the lower lip in a half circle. This seems to be the favorite place of attachment for the immature stages on the cotton rat. Unfed adults failed to attach when placed on rats.

AMBLYOMMA NAPONENSE Packard

Taken from an anteater, *T. t. chiriquensis,* at Alhajuela on the Chagras River, and also from a collared peccary, *Pecari angulatus bangsi* Goldman, in the Boqueron River region by Dr. H. C. Clark.

AMBLYOMMA OVALE Koch

Specimens of this tick were taken from an anteater, *T. t. chiriquensis*, at Alhajuela by Dr. H. C. Clark.

In the regions of the Pequeni, Boqueron and upper Chagras rivers, the ticks are so numerous in the dry season that to travel very far in the bush, or jungle, means to be literally swarmed with them. Some of the natives who spend much time in the bush, hunting, seeking rubber, etc., in this region have various methods, conceived from their own ideas, of freeing themselves from these pests. Rum with tobacco soaked in it is sometimes used to rub over the body and a ball of beeswax is also used for removing unattached larvae. As these are small and not easily picked off and destroyed by hand, the beeswax is quickly dabbed about on the body wherever they are present, thus causing them to stick to the wax. After each collection the ball is rolled between the hands until the larvae are worked deeply into the wax and subsequently die.

While there are undoubtedly many natural enemies of the ticks in Panama the most noticeable one is the groove-billed ani, *Crotophaga sulcirostris* Swains. This bird frequents the open fields and areas of sparse forest growth in the lowlands, being very numerous in the savannas, or open, grassy areas, near the Pacific coast. Many cattle are pastured on these savannas and these birds are usually in attendance busily engaged in picking the ticks from them.

Much credit is due Drs. S. T. Darling and H. C. Clark, whose names frequently appear in this article, for their zealous work in collecting ticks. During Dr. Clark's frequent hunting trips he painstakingly collected specimens from all infested animals killed and it is through his efforts that many of the species enumerated were obtained. I also wish to express my thanks and appreciation to Mr. F. C. Bishopp, of Dallas, Texas, for his kindness in identifying specimens for me.

RESÚMEN

Las garrapatas adquieren mayor importancia cada día como agentes de las enfermedades del hombre y de los animales. En

Panamá las garrapatas son numerosas y están muy esparcidas y se conocen ciertas enfermedades transmitidas por ellas. Sigue a continuación una lista y descripción de las especies estudiadas por el autor durante su estadía como entomólogo en el Laboratorio de Sanidad de Ancón:

Argas miniatus Koch

Ataca a las gallinas y otras aves domésticas. Muy abundante en Panamá. Transmite el *Spironema gallinarum,* causa de la espiroquetosis de gallinas.

Ornithodoros talaje Guerin

La garrapata humana. Muy común en Panamá. Se alimenta de noche y se oculta en las rendijas y grietas durante el día. La rata es huésped de la forma larval. Transmite el organismo de la fiebre recurrente de Panamá al hombre según se ha demostrado experimentalmente.

Dermacentor nitens Neumann

La garrapata de caballos tropical. Abundante en todas partes de Panamá en donde haya caballos. Se agarra especialmente sobre la parte interna de las orejas. Una sola hembra puede depositar tantos como 2400 huevos. La evidencia parece demostrar, aunque no se ha demostrado definitivamente, que esta garrapata transmite la piroplasmosis equina en Panamá.

Rhipicephalus sanguineus Latreille

La garrapata del perro. Se les encuentra entre los dedos y dentro de las orejas. Transmite el *piroplasma canis.* La mosca de América (*Lucilia carnicera*) deposita sus huevos a veces en las lesiones causadas por estas garrapatas.

Ixodes ricinus Linnaeus

La garrapata de las semillas del ricino. En Panamá se encuentra sólo un especimen. En Europa se la acusa de transmitir la *piroplasmosis bovina.*

Margaropus annulatus (Say)

La garrapata del ganado Norte Americano. Transmite la fiebre del ganado de Tejas. Se encuentra pero no es común en Panamá.

Margaropus annulatus australus (Fuller)

La garrapata de ganado Australiano. Muy abundante en Panamá la que infesta casi todo el ganado. Transmite la *Babesia bigemina,* infección muy común del ganado nativo.

Haemaphysalis leporis-palustris Packard

La garrapata del conejo. Se encuentra pero no es común en Panamá.

Haemaphysalis kochi Aragao

La garrapata del venado.

Amblyoma cajennense Fabricius

La garrapata Cayenne. La mas abundante, mayor distribuída y la más molestosa de las garrapatas de Panamá. Ataca a los hombres y a los animales con la misma avidez. No se sabe que tome parte en la transmisión de enfermedades infecciosas pero puede que sea activa en la distribución de huevos de la *Dermatobia hominis* Gmelin.

Amblyomma darlingi Nuttall

Se parece mucho a la *Amblyomma cajennese.* Muy común y tiene una variedad de huéspedes. No se sabe definitamente que ataque al hombre.

Amblyomma americanum Linnaeus

La garrapata de la Estrella Solitaria. Se encontraron cuatro especímenes en una isla de la bahía de Panama.

Amblyomma dissimile Koch

La garrapata de la iguana. Infesta a muchos de los animales de sangre fría, incluyendo las culebras, sapos y las iguanas. A una hembra se le observó depositar 9000 huevos en un perídodo de 35 días.

Amblyomma longirostre Koch

La garrapata del puerco espín.

Amblyomma nodosum Neumann

La garrapata del oso hormiguero.

Amblyomma varium Koch

Infesta al perezoso de dos dedos. A una hembra se le observó depositar cerca de 10,000 huevos en un período de 14 días.

Amblyomma humerale Koch

Cuatro especímenes se encontraron en tortugas.

Amblyomma parvum Aragao

La garrapata del venado. También se las encuentra en las ratas del algodón.

Amblyomma naponense Packard

Se encontraron especímenes en el oso hormiguero y en el pecarí.

Amblyomma ovale Koch

Se encontraron especímenes en el oso hormiguero.

Los nativos echan tabaco dentro de rom hasta empaparse bien y se untan el cuerpo con esto para librarse de las garrapatas. El frotarse el cuerpo con una pelota de cera sobre la superficie del cuerpo hace que las larvas despegadas se adhieran a la cera. La cera se hace rodar entonces entre las manos, de manera que las larvas se aprisionen dentro de la cera y mueran. Ciertos pájaros se alimentan con estas garrapatas que obtienen picotando al ganado infestado con ellas.

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NOTES ON YELLOW FEVER IN MEXICO[1]

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It is my belief that yellow fever was present in Mexico long before the coming of the Spaniards, and that there have been in recent years at least three areas in which this disease has been to all intents and purposes, permanently endemic, namely, Yucatán, Vera Cruz, and Colima. Probably there have been other endemic and pseudo-endemic areas. In this class I would place Campeche and Tabasco as centers which were at one time very active foci of infection, but from which the disease eliminated itself as the decadence of their commerce caused a migration of the population, thus bringing into play the principle of the failure of the human host. This principle was first developed and announced by Carter and has been of inestimable aid to the epidemiologist in shaping campaigns to eradicate yellow fever.

Yellow fever when endemic is usually to be found in a large city and is most active in a certain section of that city, a characteristic which impels me to apply to the infection the term "place disease," but with a meaning somewhat wider than was originally intended by Carter. In Mexico there are sections along certain river fronts where the homes of one community are practically continuous with those of the adjoining community. In a region of this type the center of yellow-fever endemicity will shift from time to time, and the section must, therefore, from the standpoint of the epidemiologist, be considered as one community. For this reason I have used the term "areas" in defining what I consider to have been the yellow-fever foci in

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Mexico in recent years. As a concrete illustration of my point I would cite the Vera Cruz and Yucatán areas.

The city of Vera Cruz has apparently at times been an endemic focus of the infection, especially when it was receiving cases from Havana and Mérida. This center was freed of the disease by the brilliant efforts of Dr. Licéaga, and remained clean for many years; yet, during the period when no yellow fever could be found in the city, it was present in the nearby valley of the Papaloapan River, and centered in Tuxtepec. Thus while Vera Cruz was once an endemic yellow-fever center it was not permanently so, whereas the area was.

In Yucatán the Maya records leave little doubt as to the presence of the disease there prior to the coming of the Spaniards and before the founding of Mérida, which later became the most permanent endemic center in all Mexico. While the earlier center, or centers, of endemicity remained probably in Ticul for a time after the founding of Mérida, the disease was eventually eliminated from these foci through failure of the human host, and there is no evidence that they ever again became reinfected.

The Colima area has always been circumscribed. In all likelihood yellow fever was introduced there in the earlier years before the advent of the railroads, via Manzanillo by sailing vessels from Panamá and Guayaquil, but in recent years the infection most probably came from the Vera Cruz area by way of the railroad and Salina Cruz. In Colima the disease tends to eliminate itself because of the relative smallness of the area and the absence of a very active influx of non-immunes. But as long as any infection is present there, it will spread throughout the Pacific coast region by land routes and by way of Manzanillo to other port towns, and thence into the interior. In these secondary centers where there is little movement of population, the disease will eventually disappear through failure of the human host.

NO EVIDENCE OF YELLOW FEVER ENDEMICITY ON MEXICAN PLANTATIONS

Nowhere in Mexico could I find evidence of true hacienda endemicity, nor does it appear that it has existed in recent years.

On the other hand, the records are replete with proof that haciendas have been infected yearly for consecutive years, and always the number of cases has borne a direct relation to the condition of the crops. When crops are abundant there will of necessity be an increase of laborers for harvesting, and with both non-immunes and yellow fever present, the natural consequence is an increase in the number of cases of the disease. Haciendas in line of travel from an endemic center have been repeatedly infected, and frequently these infections have assumed the proportions of an epidemic; but in no instance has the disease become endemic. The nearest approach to hacienda endemicity was probably in Yucatán. Here the only crop, henequén, is continuous the year round. Demands for this product are fairly constant in the world's markets, and therefore the demand for labor to gather the crop is also constant. This labor cannot in normal times be supplied entirely by the natives, and the additional demand is met by bringing into the area upland people, all of whom are non-immune to yellow fever. The average hacienda maintains, however, a fairly constant permanent labor force, and the survivors of yellow-fever attacks become in time fairly numerous, forming an immune wall for the newcomers, including the children. On the henequén plantations as in other industries, years of crisis were experienced in which no labor was imported. In these years the yellow-fever cases were fewer, and if the depression continued long enough, the infection was eliminated through failure of the human host.

ECONOMIC DEPRESSION A FACTOR IN YELLOW FEVER ELIMINATION

A similar principle undoubtedly operated time and again in Mexico. I am inclined to believe that Mérida freed itself of yellow fever in this way between 1918 and 1920. We know that Campeche, Tabasco, and other centers suffered from yellow fever in the days when they were commercially important, and that when the commerce they enjoyed was transferred to other places, the number of yellow-fever cases diminished, and in due time completely disappeared. We have witnessed the self-elimination of the disease from many of the islands in the Caribbean Sea when the sugar industry failed. Thus throughout the history of yellow fever the disease has marched side by side with the commerce of a locality and has disappeared only with commercial decadence or with the introduction of sanitation.

CONTROL OF YELLOW FEVER THROUGH SANITATION

The campaign to control yellow fever in Cuba extended over a period of eighteen months before it was decided that the infection had been eradicated. Dr. White checked the 1905 epidemic in New Orleans in thirty days. The Panamá campaign saw its last case in May 1906 some eighteen months after the work had actually started. The Guayaquil campaign recorded its last case six months after the work had been inaugurated.

Except in the Guayaquil campaign, fumigation, isolation of yellow-fever patients, and screening of water-containers were the principal measures practised. In Guayaquil, on Carter's suggestion, anti-larvae measures alone were practised for the first time in a large endemic yellow-fever center. Now these measures form the basis of all campaigns for the control of the infection.

In combating yellow fever the hygienist has a tremendous advantage in that the infection is not a carrying disease as is, for example, typhoid fever. The causal organism, the *Leptospira icteroides*, is destroyed in the human body after fulfilling its function. There are no known reservoirs of infection other than man and the *Aedes calopus* mosquito. The germ can be transmitted from the sick to the well only through the medium of this single species of mosquito, and the transmission can be effected only during the first three days of the disease; for after the third day the germ is not to be found in the patient's blood stream.[2]

The eradication of yellow fever is achieved when the last *Leptospira icteroides* has been destroyed. This is most rapidly accomplished, in the light of present knowledge, by the reduction of *Aedes calopus* to a theoretical minimum called the safety index, which is reached, as was shown in the Guayaquil campaign, when only 10 per cent of the fresh-water deposits are *Aedes calopus* breeding-places. Carter uses the term "critical-number period" for a stage in the control of *Aedes calopus*, which I take to mean the first marked interval in an endemic or epidemic yellow-fever center in which the area is free of the disease as a result of control measures. At this stage, as I understand it, if a case of yellow fever is introduced into the community, secondary cases may arise therefrom. When the safety index is reached, on the other hand, there is no danger from the introduction of several cases of the disease. In brief there are fewer mosquitoes available for transmitting the leptospira at the safety-index period than at the critical-number period.

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[2] These statements are based in part on the results of laboratory experiments and in part on experiences of clinicians. They appear to be substantiated by the results of the campaigns in Cuba, New Orleans, Vera Cruz, Rio de Janeiro, Guayaquil, Peru, and now in Mexico.

The destruction of the mosquito is most easily brought about by preventing its offspring from reaching the adult stage, and this is accomplished most economically by stocking with larvae-consuming fish the water containers which the mosquito selects for depositing her eggs.

HOW THE DISEASE HAS BEEN DISSEMINATED IN MEXICO

In the days before the Spanish occupation, yellow fever was spread through Mexico along the trade routes and in the wake of conquering bands that came from the plateau to make war on the coast Indians. That the Aztecs knew yellow fever is plainly shown in their picture writings describing the blood-vomit disease. These upland peoples did not of course experience the infection in their natal territory, but when they made invasion of the coast region they encountered it and reported the fact in their chronicles, which are still preserved in the National Museum.

With the advent of the Spaniards, the sailing vessel came into general use along the coast of Mexico as well as between this country and the Spanish settlements in Santa Domingo and Cuba, and unquestionably yellow fever was carried back and forth between the ports at which these boats touched; for the sailing vessel afforded an excellent breeding-place for mosquitoes, and these became infected from cases of yellow fever on board. Steamships are equipped with covered fresh-water containers and do not serve as mosquito breeding-grounds. These vessels did, however, increase the number of cases of yellow fever brought into port from short voyages. In other words, steam vessels may carry the disease, but do not ordinarily become carriers of the intermediary host of the causative germ, and therefore yellow fever eliminates itself from these vessels on long voyages.

There undoubtedly was a period in which a vicious circle was operating between Havana, Progreso, and Vera Cruz. This circle was broken only with the elimination of the disease from Cuba. Later the breach was made wider following Dr. Licéaga's successful campaign in Vera Cruz, thus leaving only Mérida, via Progreso, and possibly Tuxtepec, as the sources of infection. I am quite sure that even after the elimination of yellow fever from the city of Vera Cruz, the area still remained endemic, and that the focus of infection was in Tuxtepec.

The city of Tuxtepec is at the head of the Papoloapan valley and is the upper commercial center of the valley. Through it came the laborers from the Oaxacan hills bound for haciendas further down the valley; through it came also the products to be sent down the river to the old-time port of Tlacotálpan, which in the heyday of its prosperity was probably an additional endemic center of yellow fever. What I wish to emphasize, however, is that the valley region of the Papaloapan River was for many years infected, first in one section, then in another, and that in all probability the principal focus of infection was in Tuxtepec.

The coming of the railroad into this valley provided means for reaching the city of Vera Cruz more quickly, thus facilitating the reinfection of that city and diverting to it cases of yellow fever which in other days would have proceeded down the river, infecting towns and haciendas, until the one-time port of entry, Tlacotálpan, was reached. The latter city in all probability was at least a pseudo–endemic center. Its abandonment as a port of entry in favor of Alvarado was an important factor in freeing it of yellow fever.

In recent years the disease has unquestionably been disseminated by travelers and to a greater degree by soldiers. With the network of railways reaching to nearly all parts of the country, and the numerous coastwise steamcraft, it has been quite easy for an infected person to journey from Mérida in the south to the Rio Bravo in the north, or from the Atlantic to the Pacific Coast, well within the infective period, and ordinarily within the incubation period of the disease. Travel by horse or by foot has also been a factor in the spread of the disease, for it permits an infected person to journey by easy stages, infecting first one town, then another, until in a relatively short space of time the seeds of infection are distributed to distant parts. The avenues by which the disease can be spread are clear, and there is no difficulty in explaining its presence in any part of the country.

Whenever yellow fever is present in Mexico the country must, because of its excellent transportation facilities, be treated as one area to 1000 meters above sea-level. Experience has proved that a campaign against the disease need not be concerned with *Aedes Calopus* above the 1000-meter level; there they will not be a factor.

THE BABY AS A FACTOR IN PERPETUATING YELLOW FEVER

The report of the French Yellow Fever Commission appears to stress the importance of babies as the connecting link in maintaining endemicity of yellow fever in large cities. Undeniably babies are non-immune newcomers into an area; for we have ample proof that the immunity gained by parents cannot be transmitted to their offspring. If yellow fever were not a place disease, therefore, this element of the population would undoubtedly be a tremendous factor in maintaining active infection; but as it is a place disease, I cannot see how children in the first three years of life can play a vital part in perpetuating it; for they do not, during this time go outside of the houses as often as adults, and in the homes of an endemic center they are in many instances protected by the immunity of their parents.

The center of yellow-fever endemicity in a city is in the commercial section. Here the buildings adjoin without intervening space, and the *Aedes calopus* density is high because here are situated the better-class homes supplied with large permanent fresh-water containers, safe breeding-places which the *Aedes calopus* selects by preference for the deposit of her eggs. The families of this section are not generally as large as those of the poorer districts, and the babies cannot, therefore, be an important factor in perpetuating yellow fever. The poorer inhabitants, who have large families, live on the outskirts of the city. They cannot usually afford more than a tin or barrel for storing water, and in such receptacles the mosquito has less chance of survival than in the permanent tanks. Therefore in the districts where the number of babies is greatest, the mosquito index is apt to be fairly low.

An additional argument against considering babies an important factor in perpetuating yellow fever is the fact that a city in which the yearly number of births is large enough to maintain yellow-fever endemicity, will in all probability be sufficiently progressive to have a more sanitary system of water supply than the well, barrel, or cistern, and will, in consequence, have little or no mosquito problem.

The fact that the death-rate among babies and young children increases soon after the rainy season has started, and that this increase is coincident with an increase in the number of *Aedes calopus*, has given rise to the belief that the mortality is due to yellow fever. My belief on this point is that the mixing of the old and the new water is responsible in large measure for the high death-rate, possibly through causing a fatal gastro-enteritis. I have observed that the infant mortality decreases markedly following the stocking of water containers with fish, probably because the fish consume the bird and animal deposits washed from the roofs into the cisterns or tanks.

WHY AEDES CALOPUS INCREASE WITH THE RAINY SEASON

We have all noted an increase of *Aedes calopus* mosquitoes during the early days of the rainy season, and have assumed that this was the result of an increase in the number of breeding-places. I am inclined to think, however, that the rise in the *Aedes calopus* index with the onset of the rain is due not to the formation of additional breeding-places, but to the fact that the eggs which during the dry season were deposited in the containers just above the water-line are washed down into the tank by the rain. If active work is carried on at this time throughout the area, there should not be any actual increase in the number of mosquitoes or in the number of breeding-places. It should also be noted that in the rainy season the containers will be invaded by the Culex mosquito in greater numbers than in the dry season. This is natural, as the numerous pools formed by the rains liberate thousands of Culices. White and Hanson found in one community in Peru that all the mosquitoes in the fresh water containers were Culex. Their work had eliminated the *Aedes calopus*, and its place had been taken by the Culex.

METHODS OF CAMPAIGNING AGAINST YELLOW FEVER

Before a general campaign of yellow-fever control is begun in any locality, an epidemiological survey should be made of the infected and the infectible areas, for without such studies the field director is for all practical purposes working in the dark. A similar survey should be made before the close of operations, as it may happen that a city thought to have been an endemic center was in reality only pseudo-endemic and was receiving its infection from an adjoining area, which may not have been included in the campaign.

Control methods have undergone a radical change since the days of the first efforts in Cuba. At that time the campaign measures included fumigation, isolation of yellow-fever patients, and general reduction of mosquitoes by screening and oiling all fresh-water receptacles in use; that is, both human and insect prophylaxis was practised. For many years this program, modified to meet special local conditions, was followed in all control campaigns. Within the past few years, however, in accordance with Carter's recommendation, fumigation and the isolation of patients have been superseded by strictly anti-larval measures.

The introduction of fish as consumers of larvae has given the hygienist a measure that has proved successful in fresh-water containers; that is, it has proved successful, whenever the variety of fish most suitable for the particular container has been employed. For receptacles such as cisterns, barrels, and wells, the bottom-feeding fish has in my experience been eminently satisfactory; the top-feeding fish, Gambusia, has been equally satisfactory in fountains, but has failed in other containers. Tanks, casks, and similar receptacles are most successfully and economically protected against the mosquito when hermetically covered and sealed. If it were possible for every community to install a system of water mains that would deliver an adequate supply of potable water day and night, yellow fever would automatically disappear.

The first tangible results of a well-directed campaign to control yellow fever will be seen in about six weeks after the inauguration of the work. All efforts from this time on are for the purpose of making secure these results. Operations may be closed whenever the epidemiological factors indicate that the disease has been conquered, and it is safe to suppose that this end has been achieved when the deposit index of breeding has reached a level of 10 per cent and does not remain above this figure for any considerable time. The minimum period during which an area should be free of yellow fever before control measures may be safely relaxed is, at this time, fixed at ten months.

In closing I wish to protest against the term "sporadic yellow fever" as improper. A disease with distinct dependence upon two positive factors and which cannot possibly exist without the conjunction of both these factors does not come within the meaning of this word.

SUMARIO

Después de detalladas consideraciones sobre la epidemiología y medios de diseminación de la fiebre amarilla, el autor recomienda los siguientes métodos de campaña en contra de la enfermedad: "Antes de dar comienzo a un plan de campaña debe hacerse

una investigación epidemiológica de las areas infectadas e infectables, de le contrarie, el director de la campaña se encontrará sin puntos de partida prácticos. Una investigación parecida debe llevarse a cabo también antes de la terminación de las operaciones sanitarias pues podría ocurrir que una ciudad con apariencias de ser centro endémico sea en realidad pseudoendémica y reciba la infección de un area adyacente que puede haber sido excluída del campo de acción.

Los métodos de "control" han cambiado radicalmente desde los tiempos de los primeros esfuerzos experimentales en Cuba. Entonces, las medidas que se tomaban incluían la fumigación, aislamiento de los pacientes, reducción de número de mosquitos por medio del alambrado y uso de petroleo en los receptáculos de agua fresca. Durante varios años este programa, con modificaciones de acuerdo con la localidad, se ha ejecutado en todas las campañas. En años recientes, sin embargo, y de acuerdo con las recomendaciones de Carter, la fumigación y aislamiento han sido reemplazados por medidas de estricta destrucción de las larvas.

El uso de pececillos como consumidores de larvas en los depósitos de agua fresca, ha sido de mucha utilidad al higienista; es decir, ha tenido éxito siempre que la variedad de peces propios para el caso han sido empleados. Para tales recipientes como cisternas, barriles y pozos, los llamados peces "de fondo" son eminentemente satisfactorios. Los de "superficie," especie *Gambusia*, son igualmente satisfactorios en fuentes, pero no en otra clase de recipientes. Los tanques, toneles y otros receptáculos parecidos, se protegen, con economía y éxito, cubriéndolos y cerrándolos hermeticamente. Si a todas las comunidades les fuera posible establecer un sistema de cañerías maestras que les surtiera de día y noche con agua potable, la fiebre amarilla desaparecería automáticamente.

Los primeros resultados tangibles de una bien dirigida campaña en contra de esta enfermedad deberán observarse a las seis semanas de haber sido inaugurado el trabajo. De alli en adelante todo los empeños son tendentes a conservar los resultados obtenidos. Las operaciones se dan por terminadas sempre que los factores epidemiológicos indiquen que la enfermedad ha sido conquistada. Es cierto suponer que el objeto ha sido conseguido cuando el índice de densidad de los criaderos de mosquitos alcanza un nivel del 10% sin que ese límite se traspase por un tiempo considerable. Considero que la intensidad del trabajo debe continuar sin tregua ni mitigación por un período mínimo de diez meses.

Para terminar protesto del término corriente "fiebre amarilla esporádica" por considerarlo muy impropio. Una enfermedad como esta que depende de dos factores positivos y que no puede existir sin la conjunción de ambos, no se reduce al significado de esta expresión."

THE PATHOLOGY OF LEISHMANIOSIS OF THE NOSE

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Leishmaniosis of the skin, giving rise to characteristic and very chronic ulcers on the forearms and legs is a very well-known disease in many portions of tropical South America. The lesions usually appear singly upon the exposed surfaces of the semiclad laborers, but at other times multiple ulcers make their appearance, either in close relation with each other or, more rarely, widely scattered over the various parts of the body. When multiple ulcers are found, the individual ones are small and do not seem to progress locally. The lesion is a granuloma produced by the *Leishmania americana* which resembles closely the parasite of the oriental boil.

For many years there have been observed in divers parts of South America, ulcers of the skin and mucous membranes whose clinical descriptions bore considerable similarity, and whose differential characters have even up to the present not been clearly defined. As these ulcers usually appeared upon the skin surfaces of the laborers working in rural districts, their cause has been variously ascribed to local injuries, poisonous plants or to the bites of insects. Furthermore, as these lesions occurred in small districts or endemic foci, local names were usually given, which has led to much confusion.

In Peru it is claimed that the leishmaniosis of the skin, spoken of as uta, was an endemic disease amongst the natives more than four centuries ago and that pictorial representations of the lesions can be found upon the pottery of the Incas. This may or may not be, but from the advent of the first pioneer through certain isolated valleys, we have recurrent reports of similar skin ulcers arising under conditions similar to the present. The first report of the disease in Brazil was made by Fray Hipolito in 1827. Since this time we have reports of the sporadic occurrence of leishmaniosis in many districts from Central America to Argentina. It was Alexandre Cerqueira in 1885 and confirmed by Juliano Moreira in 1895, who identified the disease in the vicinity of Bahia and recognized the similarity with the "botão do oriente." Darling and Connor observed a case of leishmaniosis in Panama; Seidelin found the lesion in the native Indians of Yucatan, the ulcers appearing upon the ears; Neiva and Barbara found cases in the Argentine, while others have reported similar granulomata of the skin in patients in Dutch Guiana, Paraguay, Peru and Bolivia.

However, the disease appears most prevalent in Brazil, many cases being observed in the vicinity of Bahia and the upper Amazon, some occur in the State of Minas, Matto Grosso, Goyaz and the Federal District of Rio de Janeiro, and many in the State of São Paulo. The distribution is regional, often being located in the valleys of certain rivers. In São Paulo the watershed of the river Tieté has been prominent in the number of cases developing within a limited marginal zone. Other zones are also found along other rivers and valleys, such as along the Rio Pardo and the Rio Paranapanema. It is interesting to note that the disease has been accidentally transferred to some new districts and has given rise to new focal endemic centres.

In the earlier literature some confusion exists in the incomplete differentiation of leishmaniosis from other chronic skin ulcers such as may be seen in syphilis, framboesia and blastomycosis. This confusion was still further accentuated by the misapplication of names. In the north, the disease went by the name of "botão da Bahia," while in French Guiana it was called "pianbois," in Dutch Guiana "boschyaws," in British Guiana "forest yaws," in São Paulo "ulcera de Baurú," "ferida brava" and occasionally in the interior of Brazil, it was called "bouba," a word which is regularly used for all chronic ulcers of the skin. This latter term particularly has caused much heated discusssion, for it was originally applied to the lesions of framboesia. But as Breda in 1895 used the term "buba" to indicate the lesions of leishmaniosis, which he had the good fortune to study in sixteen Italians who had returned from Brazil, many careless authors have interchanged the terms until today they have become valueless and must be dropped. As recently as February, 1922, we find Moss and Bigelow reporting upon framboesia in the Dominican Republic, using the name "buba" for the disease. Furthermore, in Portuguese the word "bubão" is used for syphilitic buboes or chancres.

In Brazil, and particularly in the state of São Paulo, the discovery and the study of endemic foci of this disease were associated with the cutting down of virgin forest. When the Noroeste Railway was projected through the western portion of the state, as it followed the left bank of the Tieté, several hundred cases of leishmaniosis were sent to Santa Casa Hospital, São Paulo. Reports upon these cases have been made by Lindenberg, Carini and Paranhos, Pedroso and others. The lesions were almost always upon the ankle or leg and occasionally upon the forearm. These men were mostly axemen and laborers cleaning the right of way for the railroad. Hunters and Indians also acquired the disease in the same region. A similar history of the development of an endemic center may be found in another region, lying southeasterly from Baurú, and following the course of the Sorocabana Railway. Since the beginning of these centers of infection there have been, year after year, scores of cases applying for treatment at the hospitals of São Paulo. Occasionally the increase in the number of cases at a given center assumed epidemic proportions.

The lesion begins as a localized area of erythema and induration of the skin, and at first may resemble the bite of an insect. After some days a single nodule makes its appearance, having a smooth skin covering, painless to pressure, and eventually appearing somewhat softened in the center. A considerable necrosis takes place in the subcutaneous tissues until the overlying skin gradually is destroyed from below and eventually gives way in the central portion of the nodule. A purulent material escapes from the broken surface and the overhanging skin rapidly necroses to give rise to an open ulcer. At the beginning the skin edges are undermined, but the free borders soon recede to reach the limit of the necrosing process under the skin. The ulcerating area continues to widen, progressing slowly and then becoming stationary. Commonly the ulcer measures from 2 to 6 cm. in diameter. The borders of the ulcer are clear cut, not undermined, with little or no evidence that the subcutaneous lesion extends beyond the borders of the ulcer. The margins of the ulcer are but slightly raised and the border of induration is narrow. The ulcer is usually round, but at times the shape is distorted by an extension of the lesion in one direction. These unsymmetrical extensions suggest the progression of the infection along lymphatic channels. In the early stages, the ulcers may show quite a luxuriant development of granulation tissue, which makes an unsuccessful attempt to repair the ulcer, and occasionally the epithelium grows in from the edges, covering a margin with new white skin. Most of these attempts at repair are unsuccessful, and the new tissue repeatedly melts down. The untreated wounds receive more or less secondary infection on the surface, which is constantly giving off some exudate. There is, however, a smaller amount of exudate than might be expected from a wound of such size. As weeks and months pass on, the granulation tissue disappears or diminishes until the floor of the ulcer is smooth, red and clean. These punched out ulcers, with remarkably indolent inflammatory reactions, may remain unchanged in appearance for years. Following upon satisfactory medication, a thin layer of healthy granulation appears upon the scar base, and slowly the ulcer closes in from the sides. There is some similarity in the appearance of the ulcer with syphilis.

American leishmaniosis is a localized ulcerating lesion of the skin which is acquired upon the exposed parts. Often the ulcers are symmetrical on the legs or arms, or single ulcers appear upon the face or ears, seldom on the parts of the body which are covered. Although those who are affected by this disease do not take any particular care in the protection of the early ulcer, it is very unusual to find implantation ulcers in the vicinity of the original one, or even on other parts of the body. In the crude methods of dressing the wounds of workmen in the far interior, the hands cannot fail to be soiled by the secretions of the ulcer, and yet, secondary ulcers of the fingers are very rare. One does, however, see occasional cases in which new ulcers appear centrifugal from the primary one and apparently along the course of the lymphatics. When the primary ulcer has been upon the hand or foot, the lymphatics from this region may be palpated, demonstrating a chain of nodules along their course. Some of these nodules may give rise to new ulcers.

A rare type of leishmaniosis is seen in the papular variety, which, instead of developing excavated ulcers, shows curious cauliflower masses projecting above the skin. This form, it is said, persists for many years, and is resistant to treatment. The more common ulcerating type of the disease may occasionally undergo spontaneous cure. This, however, is decidedly unusual.

Pedroso has shown that the lesions are spontaneously acquired by dogs in the infected regions, usually appearing about the nose or face. Furthermore, he was able to reproduce the lesions in dogs by inoculating them with cultures and material isolated from human cases. It is not known whether some of the wild animals of the forest in the endemic centers harbor the parasite or not. Some studies upon this problem have been made in the hope that the mode of distribution and natural propagation of the parasites could be learned. This still remains obscure.

In 1909, A. Lindenberg reported upon the finding of parasites similar to those reported upon by Wright, in 1904, in a case of oriental sore. Immediately following this, the findings of Lindenberg were substantiated by Carini and Paranhos. Since then many studies have been made upon the parasite to identify it or differentiate it from *Leishmania tropica.* Minor points of differentiation have been reported, but essentially these parasites are identical. Pedroso and Dias cultivated the Brazilian leishmania on Novy's medium in 1912. Clinically the eastern and the western types of disease produced by the leishmania have many points of difference, but the parasites themselves are very similar.

One of the unique features of the American leishmaniosis is the frequency with which chronic ulceration of the arms or legs is followed by mucosal lesions of the nose, pharynx and larynx. The lesions are more frequent than is usually credited, some even claiming that all untreated skin infections by leishmania will sooner or later give rise to mucosal involvement. These make their appearance from eight months to fifteen years after the development of the initial skin ulcer, and they appear to be types of true metastatic implantations. In São Paulo the nasal and buccal lesions appear in from 15 to 20 per cent of all cases in which the skin lesions are more than two years old.

The American leishmaniosis differs in a number of respects from the Oriental infection, and although the parasites are all but indistinguishable, one striking difference lies in the frequency with which the American type develops mucosal lesions while these are seldom found in the oriental form, save as occasional primary localizations of the "Bouton d'Orient" (Laveran). There is, therefore, never any difficulty, in regions of South America where leishmaniosis is endemic, in finding cases of nasopharyngeal involvement.

Repeated studies have been made to determine the mode of transmission of the American leishmania, and although many insect types have been named as possible carriers of the parasite, no conclusive evidence has been brought forward in favor of any single one. It has been suggested that the parasite ordinarily passes its life cycle in one or more of the wild animals of the South American forests, and that man is only a chance host. Others suggest that the human infection is the result of an accidental adaptation of a parasite normally harbored by insects (Darling and Townsend). As Brumpt and Pedroso point out, there are three important points related to the appearance of leishmaniosis in São Paulo, (1) the infection is acquired in the virgin forests, (2) the ulcers appear on the exposed part of the body, and (3) the infection is more frequent in certain months of the year. These authors studied a great variety of animal parasites which could possibly act as temporary hosts in transmitting the disease to man. Although a large number of biting insects were considered in these studies, the authors were unable to determine the transmitting agent.

In 1910 Bueno de Miranda gave the first description and study of the mucosal lesions of leishmaniosis. These mucosal lesions of the nose and pharynx must be considered as secondary processes to the skin infections. Here again the mode of implantation is far from clear. It has been suggested that they represent true metastatic foci following a carriage of the parasites by the blood or lymphatic vessels. Others believe that the nasal infection is brought about through an implantation by the fingers from the open ulcers which contain a certain number of parasites in the exudate. The late appearance of the secondary lesions, often occurring at a time when the parasites are extremely scarce in the exudate of the primary ulcer, or even after the primary ulcer has cicatrized, the long interval of time, as long as five to fifteen years, intervening between the primary and secondary infection, favors the view of true metastatic infection. On the other hand, up to the present, no internal lesions have been found in American leishmaniosis, and the parasite has as yet not been discovered in the circulating blood. However, when we recollect the history of many tertiary lesions of syphilis, we have before us analogous metastatic lesions. The time period during which the infectious agent actually circulates in the blood may be very short, and the development of a new focus in a distant organ may proceed with great leisure.

In 1911, Escomel, in Peru, gave a good description of the nasal and mucosal lesions of leishmaniosis, which in that country were known as espundia.

The study of the tissue changes in leishmaniosis, demonstrating the progress of the lesions from the earliest reactions to the late ulcer and cicatrization, can be more easily followed in the nasal involvement than in the skin. It is infrequent to encounter the cutaneous lesions prior to ulceration, although small nodules of new infection are at times discovered in the vicinity of an old ulcer.

This study was undertaken to determine the nature and progress of the tissue reaction as found in the nasal mucous membrane when infected by the *Leishmania americana.* Dr. Henrique Lindenberg in whose oto-rhinological clinic, many cases of leishmaniosis of the nose, pharynx, mouth and larynx annually apply for treatment, excised small specimens for examination. The material thus gained for study represents all the stages of the disease, from the early hyperemic nodular swelling of the nasal mucosa to the late ulcerating phases in which the destruction of the nasal cartilage, alae of the nose, and deep ulceration of the pharynx are prominent features.

The nose lesion begins beneath the mucous membrane covering the cartilaginous septum. It begins within the nose and does not commence in the skin covering the nose, and seldom at the entrance of the nares. The first evidence is recognized in a slight oedematous swelling and reddening of the mucosa. The swelling slowly increases and becomes harder. The passage is partly occluded. Ulcers then appear on the surface of the septum and a purulent discharge incrusts the involved part. The ulcer then spreads in its periphery, extending backwards to the palate and pharyngeal wall, as well as downwards to the nasal opening. The progressive ulceration is accompanied by considerable destruction of the deep tissues so that the cartilaginous septum becomes perforated, the alae of the nose eroded, and the palate scarred and distorted. Much deformity may thus be induced.

The progress of the ulcers is slow, usually a matter of some years. The extension into the pharynx may reach as far as the larynx, destroying portions of the epiglottis as well as the vocal cords. The mucosa of the mouth, tongue and fauces may also become involved. During the progress of the nasal and pharyngeal ulceration there may be little influence upon the general health of the patient. Fatal cases result more commonly from intercurrent diseases, particularly from broncho-pneumonia.

As this disease has only a rural distribution and develops in regions which are for the first time being prepared for cultivation, it is rarely seen by a physician in its early stages of development. The nasal lesions, which are almost always secondary to the skin infections, appear so long after the appearance of the primary lesion, often after it has been healed, that the patient does not associate the one with the other. Only when the secondary lesion causes serious difficulty in breathing or has caused much deformity, does he seek aid. Hence, it is very unusual to have an opportunity of studying the incipient stages of leishmaniosis, either in its primary skin lesions or its secondary mucosal lesions.

Because of the manner in which the infection is acquired, the disease is much more common in men than women. The statistics upon the incidence and distribution of the various types of leishmaniosis of Brazil are not sufficiently trustworthy to repeat. For the study of the incidence and distribution of leishmaniosis in São Paulo we would refer the reader to the thesis of Carlos da Silveira (1919).

PATHOLOGICAL HISTOLOGY

The specimens were excised from the nose and pharynx and were then immediately immersed in the fixative fluids. Various fixatives were used to be able to apply a variety of staining methods (formalin, Zenker's, Muller's, bichloride alcohol). For the demonstration of the parasites, Zenker's solution and bichloride alcohol gave the best results, care however must be taken not to over-fix the tissues, as shrinkage of the cells makes it very difficult to demonstrate the parasites. The excessive handling of the tissue in the preparation of paraffin blocks increases the difficulty of obtaining as satisfactory results as can be obtained in fresh-smear preparations. Fixation of small blocks for four to eight hours is sufficient, and the dehydration by alcohol should be as short as possible. I have had no success in demonstrating the parasites in tissues fixed by formalin. Mallory's eosin-methylene blue and Giemsa's stain serve well to give a good tissue stain and also for demonstrating the leishmania within the cells. It is better to stain longer with a weak solution of Giemsa, than to stain too strongly and try to decolorize. Three drops of Giemsa in 15 cc. of water will stain well in five hours, and relatively little decolorization will be necessary. For a study of the histological characters of the tissue, Boehmer's hematoxylin and eosin give satisfactory results.

In as much as the portions of the mucosa of the nose or pharynx are always small, care must be exercised in cutting the small blocks for section at right angles to the surface mucosa. The distribution of the inflammatory zones are not uniform at all levels, and an accurate impression cannot be gained where the mucosa is cut slanting. This is particularly true in the early lesions when it is desirable to observe the first evidence of the inflammatory response.

We have had an opportunity of studying the pathological histology of the mucosal (nasal and pharyngeal) lesions of fifteen different cases of leishmaniosis. In some of these cases we have had two or three specimens, taken from different portions of the process, representing early non-ulcerated nodules, ulcers recent or old, and erosions of the nasal cartilage. The mucosal specimens taken from the surface of the septal cartilage were necessarily thin, and the reaction was crowded between the epithelial surface and the perichondrium. The borders of the old ulcers were thickened, and in them it was difficult to recognize the normal disposition of the layers, and the relation of the inflammatory processes to the contained vessels and mucous glands.

EARLY STAGE—NON-ULCERATED NODULES OF MUCOSA

These non-ulcerated nodules were secondary elevations developing in the vicinity of more severe lesions in the nose. It is almost impossible to obtain the primary non-ulcerated lesions of the nose, as usually at the time when the patient seeks aid, ulceration has already taken place. However, in those cases with definite lesions on the nasal septum, search will often reveal a number of developing nodules on other portions of the mucosa. A study of these nodules does not permit one to directly draw conclusions concerning the manner of the primary implantation of the infection in the nose, but nevertheless it does give the evidence of the possibilities of the mode of transference from one situation to another.

In the earliest lesion coming under our observation, the process was distributed in small focal areas, in the deep part of the nasal mucosa. The epithelium was intact, the submucosa was slightly oedematous, a few blood vessels were dilated, and the mucous glands were normal. Many of the deep lymphatic channels were dilated and contained more cells than normal. The focal areas of inflammation appeared along the course of the small arterioles and seemingly began in the loose tissue of the adventitia. The focal areas along the vessels not uncommonly were located amongst the mucous glands, so that at first sight it suggested a relation in the mode of infection with these structures. We have not been able to establish any connection between the presence of the leishmania and the mucous glands. The infec-

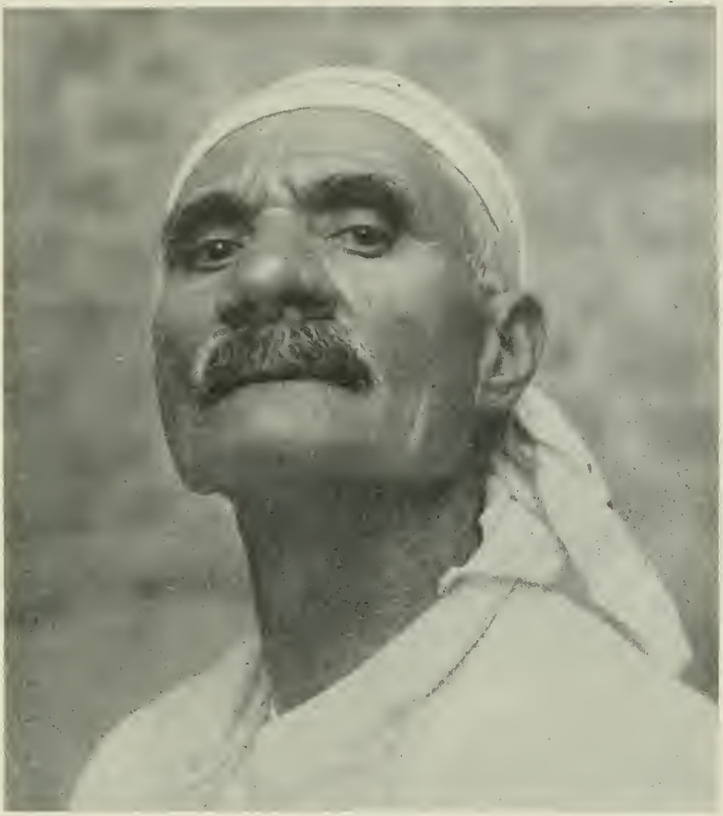

FIG. 1

tion and the inflammatory foci appeared to have a more direct relation with the perivascular lymphatics.

These early foci consisted of aggregations of lymphocytes, plasma cells, an occasional polynuclear neutrophile, and a few endothelial cells. The small lymphocyte was the predominant cell in the reaction. These inflammatory foci were loose in their arrangement and did not develop a compact mass. There was nothing unique in the arrangement of the cells. Leishmania could be demonstrated in an occasional endothelial cell, their numbers however were small, and in a single focus only one or two such cells could be found with the parasites. A few dilated

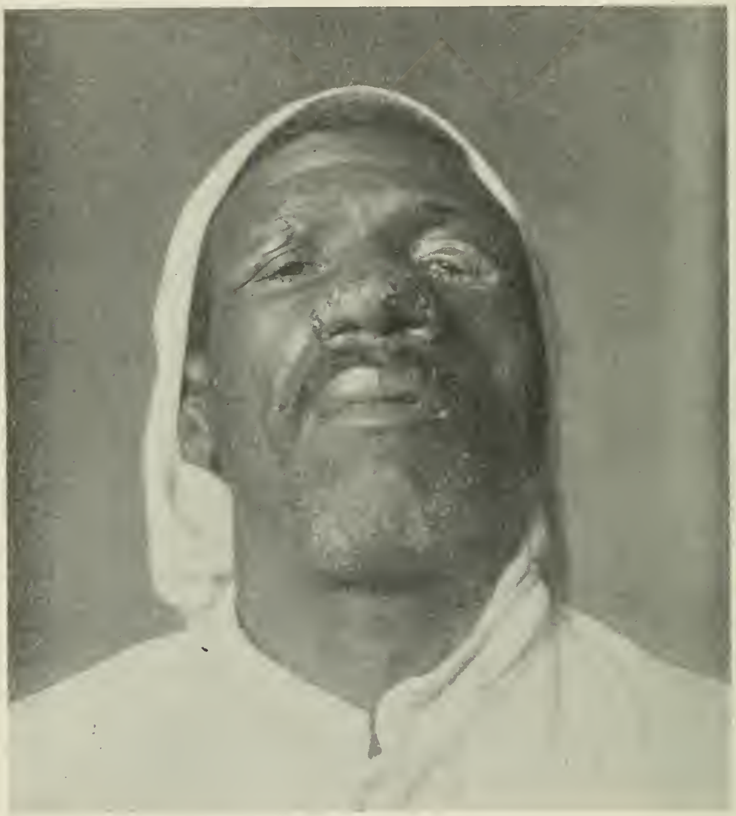

Fig. 2

lymphatics could be recognized in the vicinity of each focus, and within them were found lymphocytes and endothelial cells. In these very early reactions little change was evident in the walls of the involved blood vessels. Some small arterioles showed the presence of lymphocytes in the muscle tissue of the media but endarteritis had as yet not appeared. Small blood capillaries lying in the localized foci showed endothelial hyperplasia, an external proliferation giving rise to a crescentic double layer of cells and appearing to be one of the sources of the endothelial cells which appeared in the neighboring reaction. The blood vascular endothelium appeared to participate more actively in the reaction than that of the lymphatic channels.

SECOND STAGE—EARLY EROSION OF MUCOSA

In this stage the mucosa is more decidedly swollen, the surface is wavy and when the lesion involves the upper part of the septum and the vicinity of the turbinate bones, the single layer of cubical epithelium is found to be partly desquammated. This desquammation takes place opposite the inflammatory foci which are found advancing from the deep parts towards the surface. The epithelial layer lying opposite to the uninvolved submucosa, remains attached. Thus the loss of the surface membrane begins in a very irregular manner and is in patches. The ulcer is not developed through the presence of agents acting on the surface, but like the development of the tuberculous ulcer of the intestine, is dependent upon the progress of the pathological processes beneath the epithelial layer. The early surface lesions not uncommonly show areas of various stages of the process, some with recent desquammation, others showing desquammation, hyaline change, and early necrosis of the superficial exposed tissues, other areas showing an early exudate of fibrin and serum, while the older ones show true superficial necrosis covered by an adherent exudate of fibrin and polynuclear leucocytes.

The acute reaction is quite superficial and is not directly related to the deeper reactions caused by the leishmania.

During this period the lesions resulting from the presence of the leishmania show quite a new character. The inflammatory process is now more diffuse, the infiltrations are no longer isolated to the peri-vascular tissues, and the focal appearance of the infection is lost. The tissues show the effect of a more intense process, there is more or less oedema, the blood vessels are engorged and the swelling tends to distort the structures and to give an uneven contour to the surface. The diffuse infiltration surrounds the blood vessels, involves the lymphatics, and lies between the mucous glands. Frequently the clusters of mucous glands are separated from each other by the amount of cellular exudate. In this stage the presence of an extensive cellular infiltration is the most important feature. The character of this exudate is worthy of note, for now we find that it has changed its relative proportion of cells from what may be seen in the first stage. Now we find that the diffuse cellular infiltration consists mainly of plasma cells, with only about half the number of lymphocytes, a fair number of scattered endothelial cells, an occasional multinulceate cell and a very few fibroblasts. New blood vessels are not formed, and polynuclear leucocytes are conspicuous by their absence. An occasional tissue eosinophile is found. Only as we approach the surface in the neighborhood of the epithelial erosions and early ulcers do we encounter the polynuclear neutrophile.

The interesting feature in this stage is the diffuse cellular reaction, the presence of the large number of plasma cells, and the increasing number of endothelial cells which are scattered without definite arrangement to the other cells, through the involved tissues. The proportion of these endothelial cells differs in the different portions of the same section, but what has appeared to us of importance was the closer grouping of the endothelial cells, their continued increase in relative and actual numbers indicating an advance of the inflammatory process towards the next and more chronic stage.

In some cases the endothelial cell proliferation was unique, and occurred in an amount seldom seen in granulomas. We have one specimen in particular, showing the progressive reactionary stage of the deep tissues, and a relatively early process of ulceration on the surface. The reaction in the tissue is in largest extent of endothelial cells, while lymphocytes and plasma cells are each present in lesser numbers and in about equal proportion to each other. The endothelial cells have the appearance of those seen in Hodgkin's disease, occurring in plaques and sometimes in continuous layers with poorly defined divisions between the cells. Or again, where the endothelial cells are scattered among the lymphocytic infiltration their contour is more spherical, the cells are isolated, the cytoplasm is faintly granular and the centrally placed nucleus appears small and dark. We know of no other granulomatous process which in one stage of its development shows such extensive proliferative activity of its endothelial cells. Furthermore, amongst the aggregations of endothelial cells we encounter many multinucleate ones. These latter vary much in size and in the arrangement of the nuclei. Their tendency is to remain spherical, and we do not encounter the large stellate Langhans giant cell. In fact there are but few cytoplasmic processes extending into the surrounding tissue. These cells show a clear homogeneous cytoplasm, amphoteric in reaction, and their nuclei arranged in a very inconstant manner. At times the nuclei lie along the periphery, or they are aggregated in a cluster in the center or towards one side, or again they may be diffusely scattered through the cytoplasm.

Both the endothelial cells and their giant cells possess phagocytic properties. We have observed the presence of cell debris of lymphocytes and of the leishmania within their cytoplasm. It is in lesions of this kind, where large numbers of endothelial cells compose the tissue reaction, that the greatest number of parasites can be demonstrated in the cells. These endothelial cells contain one or more leishmania, and we have seen as many as eight in a single cell in the section. We have not seen the parasite in fibroblasts nor in neutrophile leucocytes. Occasionally parasites may be seen lying free in the tissues, but such are rare in this stage, where no necrosis is to be found, and when the tissues are crowded with endothelial cells actively phagocytic for them. New blood vessels are unusual in this reaction and fibroblasts are encountered sparsely.

In the regions of the arterioles and capillaries which form the rich vascular supply of the mucosa of the nose it is possible to study and observe the development of the inflammatory endothelial cells from their lining membranes. The origin of these cells appears to be similar to that described by Mallory for typhoid fever and measles, and by Permar and Floor for the endothelial phagocytes of the lung. Growth of the lining endothelial cells of vascular channels gives rise to new cells in the periphery which have the property of migration and of an independent existence. In our material, there was abundant evidence of such a process of growth, and we were convinced that the endothelial cells in the areas of reaction were from this source. There was also evidence, though not so clear, that the endothelial membrane of the lymphatic channels could also participate in the endothelial cell production.

THIRD STAGE—ULCERATION OF MUCOUS MEMBRANE

When lesions have advanced to this stage of the disease, much disfigurement has already occurred, destruction of the alae of the nose, the nasal septum, pharyngeal ulceration or laryngeal involvement. This stage is the most common one met with in the hospital at São Paulo. All of the cases have or have had their chronic ulcers of leishmaniosis on the legs or arms, and they do not seek aid for the nasal condition until it is well advanced. To obtain material for the earlier stages a systematic search of the nose must be made of all of the cases of skin leishmaniosis.

We have studied the ulcerated lesions, in the region of the ulcer, in the depths of the mucosa, at the border of the eroding cartilage and in regions somewhat removed from the acute superficial ulcer. In studying this stage of this peculiar granulomatous process there is one feature which immediately attracts attention, and that is, that the acute superficial process has very little relation to the deep granuloma and has no influence in modifying its progress. The ulcer on the surface, with its acute fibrinous exudate, is very superficial, and no infiltration of polynuclear leucocytes is to be found beyond a few cells in depth. The ulcer is produced through desquamation and atrophy of the epithelial cells, and not by a process of primary necrosis. The stratified epithelial layer gradually becomes thinner, and no new epithelial cells are produced in the depth of the membrane. When the surface is denuded, the surface irritation and bacterial infection cause an acute superficial reaction, usually consisting of a layer of fibrin and leucocytes. No leishmania can be demonstrated in this acute exudate, and we have not encountered neutrophile leucocytes containing leishmania.

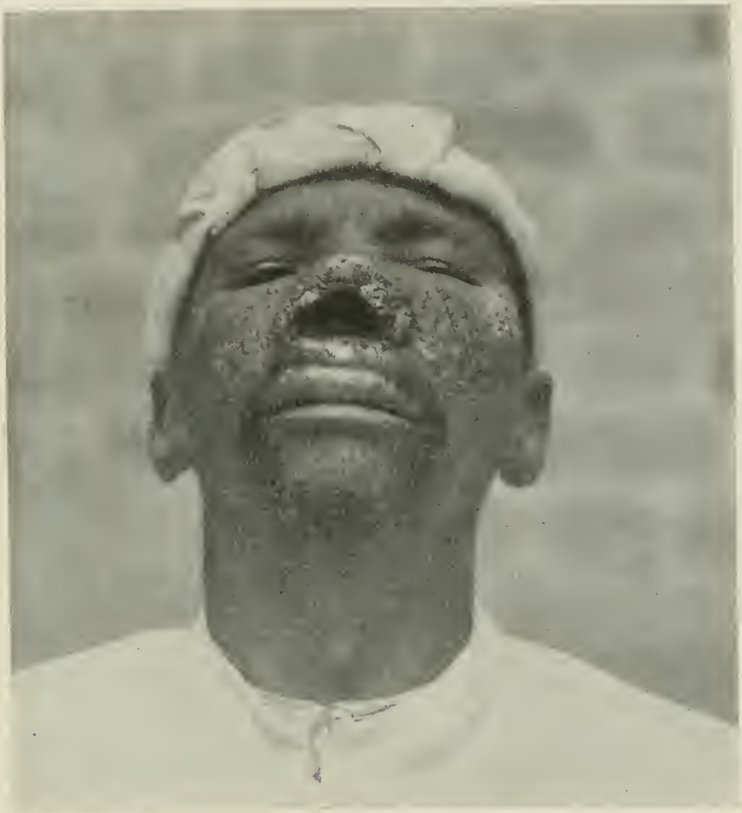

Fig. 3

The important lesions of leishmaniosis lie in the submucosa at a little distance from the surface. The character of the reaction has now taken on new qualities and differs quite decidedly from that seen in the second stage. The diffuse inflammatory lymphocytes, plasma cells and endothelial cells have now assumed a new arrangement and although the same cells are participating in this tissues response, there seems to be an attempt to dispose the cells in groups and clusters. We again find nodule formation. In this late stage of granuloma formation we are able to demonstrate nodules of all ages and of all stages of advance towards necrosis or fibrosis.

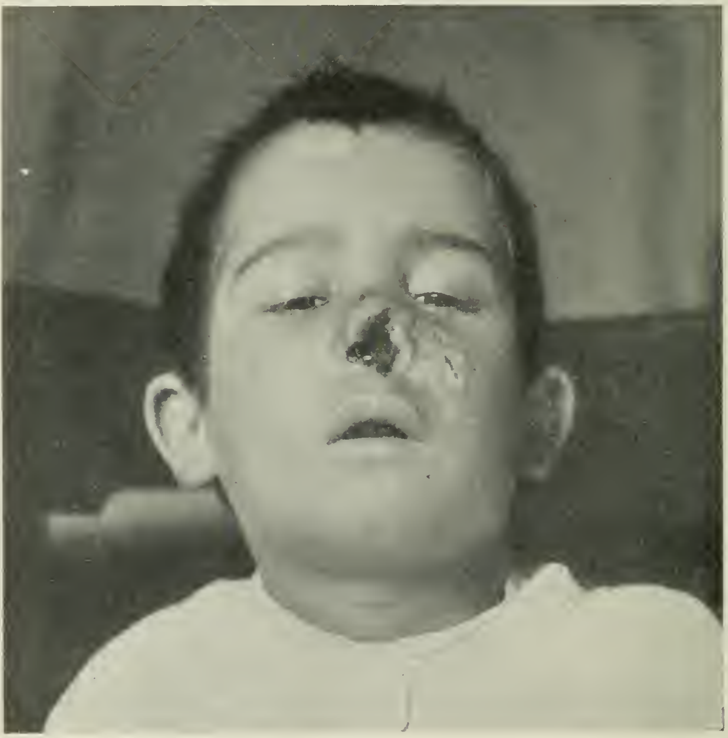

Fig. 4

Nodule formation begins with the aggregation of clusters of transparent-looking (eosin-methylene-blue) endothelial cells, surrounded by dense groups of lymphocytes and plasma cells. Amongst the endothelial groups there may be one or more giant cells and leishmania can be demonstrated in a few endothelial cells in the center of the nodule. In this stage fibroblasts are scarce. In fact at this time, the amount of stroma throughout the thickened submucosa is small, and within the endothelial clusters it is very slight or absent. These endothelial nodules are aggregated along the course of the small blood vessels. One arteriole was fortunately cut longitudinally for some distance and surrounding it were three distinct and separate nodules. At times the nodules make their appearance between the groups of mucous glands, separating them and giving the appearance of a direct association between the nodule formation and the gland structure. This, however, is only apparent as we always find that the presence of the nodule was in relation to a blood vessel which coursed between the glands. The same arrangement is to be found in the deep

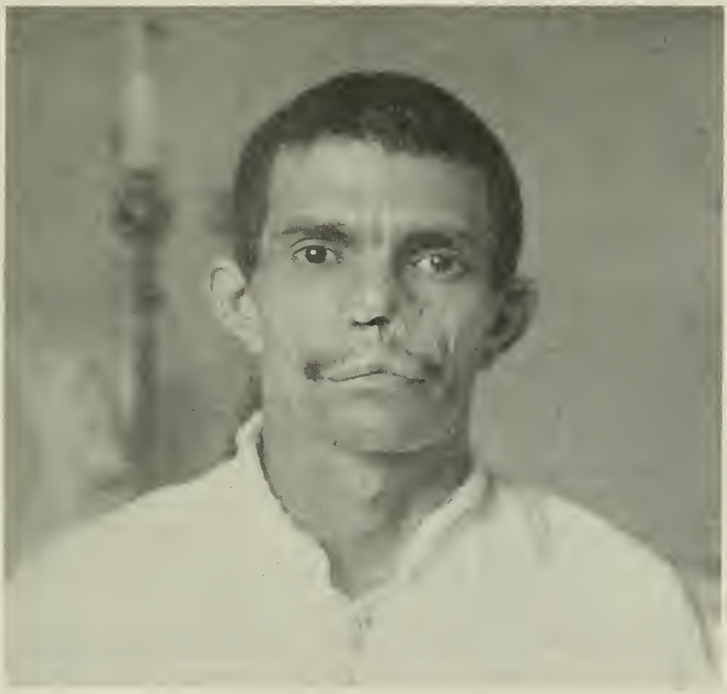

Fig. 5

tissues near the nasal cartilage. The periphery of the arterioles forms the focus for the localization of the infective process. With the development of the perivascular nodules the diffuse cellular reaction more or less disappears, and the inflammation becomes focal. The intervening tissues are cleared of the previous cellular infiltration, and again assume a more normal character. The uninvolved mucous glands again are recognized with ease, which was not possible when the entire tissues were flooded with an inflammatory infiltration. The number of nodules present varies in the different cases and even in different areas of the same case.

Nodule formation in the deep mucosa is the most prominent and important development in leishmania infection of the nose. The preceding stages are the immature granulomatous processes leading up to nodule formation, when, on account of the pronounced tissue alterations, there develops the mucosal ulceration, the irregular focal necrosis, and the gradual destruction of cartilage. The influence of the leishmania infection makes itself felt at this time, not by the systemic disturbances, but locally in its direct damage upon the area infected and the secondary or indirect influences upon the surrounding tissues.

Three types of nodules can be distinguished. These nodules differing in appearance and structure are only stages of a common process and represent different age periods of the infection. These three types are first, the endothelial nodule, second, the necrosing nodule, and third, the fibrosing nodule.

As we have previously mentioned, the beginning of the third stage differs from the reaction in the preceding phase by the development of endothelial cell aggregations which have the appearance of transparent nodules, surrounded by a diffuse infiltration of lymphocytes and plasma cells. These nodules may be numerous and may even touch each other at their borders without fusing their structures. They do not merge with one another as do contiguous tubercles. Apparently this type of nodule may remain quiescent for considerable periods of time, while the surrounding tissues lose much of the cellular infiltration and are restored to more normal appearances. These nodules are almost devoid of stroma, and there is no evidence of a growth of fibroblasts either within the nodule or at its periphery.

However, at a later period the nodule begins to change its appearance; the lymphocytes and plasma cells continue to disappear from the peripheral zones, and are to be found only in scattered patches about vessels or glands. The nodules become slightly more compact, the cells lose their transparency and are arranged either in a concentric fashion or in a loose radial structure. Granular necrosis makes its appearance in the center of the nodule, occupying a small portion, or in other nodules occupying as much as half of their extent. This necrosis has no individual character, and when fully developed leaves a pink staining debris. It does not form a compact mass as seen in caseous necrosis. When these necrosing nodules lie near the surface they are prone to become secondarily infected, and will then demonstrate an acute polynuclear reaction in their center. We have observed occasional deep nodules showing similar secondary leucocytic infiltrations. When involved in secondary infection the necrosis and tissue destruction goes on more rapidly. When a nodule is closely attached to an arteriole of fair size, we have observed that the necrosis instead of being granular shows a more homogenous character. Thus within short distances of each other may be found granular necrosis and hyaline-like necrosis. Some giant cells of different sizes and shapes are present in the periphery of the nodules. The parasites may be seen in a few cells near the center, they are not common in the periphery and it is very unusual to distinguish any in the areas of necrosis.

The fibrous nodules differ in the degree of fibrosis which permeates the area. The fibroblasts grow in from the periphery and separate the endothelial cells which now have lost their transparency and show a denser pink staining cytoplasm. The endothelial cells become crowded into smaller spaces, and are recognized with greater difficulty. The compact character of the nodule reminds one of the arrangement of the cells in old lepra nodules, with the difference that in leishmaniosis the nodules are only of microscopic size, they are sharply limited in the periphery and they are usually solitary. When carefully studied in consecutive sections the nodules will always be found surrounding a small blood vessel. These nodules appear to represent the advanced stage of chronicity. Occasionally a nodule in an earlier phase of the process may be seen in the vicinity of the older lesions. The lesions appear to be progressive. New blood vessels do not develop in association with the leishmania nodules, but they do make their appearance in the floor of the ulcer. Thus the base of the ulcer shows a granulation tissue with some new vessels pointing towards the injured surface, while at the same time the deeper portions of the floor show fibrosis and nodule formation.

Naturally, as is to be expected, the presence of granulomatous nodules about the blood vessels, produces considerable endarteritis. All degrees of tissue proliferation of the intima may be found, varying from a slight hyperplasia of the endothelial membrane, to a complete occlusion of the lumen. The endarteritis is one of the most common late pathological processes in the chronic ulcerating leishmaniosis of the nose. The complete occlusion of the vessel does not take place by the proliferating endarteritis alone, but usually a thrombosis localizes upon the endarteritis at the narrowest point of the lumen.

The development of firm scar tissue through the involved areas, the presence of progressive infected nodules, and the constant occurrence of an endarteritis, often occluding the lumen, gives a character to the submucosal tissues of the nose whereby ulceration, deformity and erosion of the nasal septum becomes a logical sequence. The vascular supply (nutrition) of the tissues becomes so reduced and obstructed that none but the coarsest connective tissue can survive. The leishmania infection has not been found to localize within the substance of the cartilage, nor does the cartilage appear to be directly influenced by the presence of the neighboring nodules of the granuloma. The influence upon the cartilage appears to be two-fold, first, the effect of a neighboring inflammation which always has a definite effect upon the matrix of the stroma tissues, and second, the influence of an altered and diminished circulation. The cartilage appears to slowly change the reaction of its matrix, and then at the periphery its substance undergoes a process of liquefaction. These changes go on even at a distance from definite leishmania nodules. When once under way, the process of cartilage destruction goes on rapidly. During these changes no evidence of the perichondrium can be found, it has become lost in the surrounding inflammatory zones, and amidst the new fibroblasts which occupy the areas deserted by the matrix of the cartilage.

It is well to add a word respecting the lymphatics, which as we have previously observed, in almost all stages of the disease participate in the general reaction. The nasal mucosa is rich in lymphatic channels which respond by dilatation, and continue to function throughout the changing events of the process. The lymphatics become carriers of excess fluid and of cells. It is very probable, although we do not have the conclusive evidence, that the lymphatic channels play an important rôle in the local spread of the disease. The continuous spread of the lesions throughout the nose and the pharynx as far as the larynx is in all probability related to the lymphatic drainage of these parts.

The most striking features of the pathological change in the tissues of the nose are: (1) the primary perivascular lymphocytic infiltration of the submucosa; (2) the gradual dominance in numbers of the plasma cells and the endothelial cells; (3) the development of endothelial nodules, which become aggregated about the vascular channels; (4) the appearance of three types of nodules, (*a*) endothelial, (*b*) necrosing, and (*c*) fibrous; (5) the presence of marked endarteritis with occlusion of many vessels, (6) the presence of the organism *Leishmania brasiliensis* in all stages of the disease, within the endothelial cells.

CONCLUSION

This report comprises a study of fifteen cases of leishmaniosis of the nose, representing all stages of the disease from early nodular swelling of the nasal mucosa to the late destruction of septum and alae. The lesion is one of a peculiar granuloma, having individual and characteristic tissue reactions, and passing through successive stages. The parasite of the disease, which can be demonstrated in the various phases of the development of the granuloma, has per se, only moderate destructive action upon the tissues. The destruction of the soft tissues and cartilage is to a great extent brought about by the progressive local endarteritis causing nutritional atrophy and necrosis. The tissue reaction due to the parasite never causes a purulent inflammation, such as occurs with blastomyces and actinomyces, and a fair amount of scar tissue is produced at the border of the involved area.

The manner of development of the lesion of the nose suggests a metastatic distribution from the primary ulcer on the arms or legs. On the other hand, the centrifugal spread from the earliest point of infection, and the microscopic character of the local process suggests a lymphatic dissemination of the immediate neighborhood.

The granuloma beginning as a perivascular lymphocytic infiltration of the submucosa, passes through stages in which the plasma cells and endothelial cells gradually gain dominance, and ends with the development of nodules largely composed of endothelial cells. These nodules may show central necrosis, or may undergo fibrosis. In the late stages, the accompanying endarteritis of the neighboring vessels leads to extensive ulceration and necrosis. The leishmania are almost constantly to be found intracellular, within endothelial cells.

SUMMARIO

Este informe comprende un estudio de quince casos de leishmaniosis de la nariz que representan todos los aspectos de la enfermedad desde la hinchazón nodular de la mucosa nasal hasta la destrucción del tabique y las alas. La lesión es algo asi como una granuloma, de reacciones características y que pasa por ciertas fases sucesivas. El parásito de la enfermedad que puede ser demostrado en varias fases de la enfermedad tiene, de por sí, una acción destructiva moderada sobre los tejidos. La destrucción de los tejidos blandos y el cartílago son ocasionados en gran manera por una endarteritis progresiva local que causa atrofia de nutrición y necrosis. La reacción en el

tejido debida al parásito nunca causa inflamación purulenta como ocurre en las blastomicosis y actinomicosis. Una cantidad de tejido cicatricial se forma en los bordes del area afectada.

El desarrollo de la lesión en la nariz sugiere una distribución metastática de las ulceras primarias en los brazos o las piernas. De otra parte, la extensión centrífuga desde el punto de infección original y el caracter microscópico del proceso local, sugieren la diseminación linfática en la vecindad inmediata.

El granuloma empieza como una infiltración limfocítica perivascular de la submucosa, pasa por ciertas fases en las cuales las células plasmáticas y endoteliales gradualmente adquieren dominio, y terminan con el desarrollo de nódulos en su generalidad compuestos de células endoteliales. Estos nódulos puede que contengan necrosis central o que se conviertan en fibrosis. En las ultimas fases la endarteritis acompañante de los vasos contíguos produce ulceración extensa y necrosis. Los leishmanias se demuestran constantemente en la region intra-celular, dentro de células endotheliales.

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BRONCHIAL SPIROCHETOSIS

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Bronchial spirochetosis is an inflammatory condition of the bronchi and alveoli caused by or at least accompanied with large numbers of spirochetes.

HISTORY

In 1905 Castellani described the disease and in 1907 he found the *Spirocheta bronchialis* and looked upon it as the cause. In 1915 Fantham published a description of the spirochete. Later investigators in Asia, Africa and Europe published short treatises on it. During the war the disease was found in Europe, probably due to troops coming from countries where the disease is prevalent.

It is found in the tropics girdling the earth, and was recently also discovered in Italy, Switzerland, France and England. Reports from only a few places in China lead us to suspect that it is not generally prevalent throughout the country. In and about Nanking, however, there are many cases and it is largely upon the experience of our clinic and in patients suffering from this disease that we base the following information. Although several articles have been written on the subject, we were able to get only very little literature for reference.

CAUSE

From the histories of our patients we believe that the predisposing cause is catching cold. Anemia and generally lowered vitality have undoubtedly also a great deal to do with the cause of the trouble. A very large per cent of the cases developed their cough after they caught cold.

The direct cause of the disease is without question the bronchial spirochete. This spirochete, like the other spirochetes, in spiral shaped. Some have 5, others have 4, while again others have as many as 8 spirals. They vary in length from 5 to 30 microns and from 0.2 to 0.6 microns in width. All sizes may occur in the same slide. In the fresh specimen we have seen movements of the spirochete. After a period of motility the spirochete develops into a coccoid state, from which the new spirochetes may develop. By laboratory methods the bronchial spirochete can be distinguished from *Spirocheta pallida* and *dentium.* It is well to remember, that, in the normal mouth, *Spirocheta buccalis* maybe found, but in all cases of true bronchial spirochetosis, the clinical picture aids in the diagnosis.

Infection by the spirochete probably takes place directly from an infected patient. Several of our nurses in the University Hospital, Nanking, came down with the disease, evidently due to contact with the patients. The disease is probably contracted as tuberculosis by the droplet method.

VARIETIES

Two clinical varieties of the disease are fairly easily distinguished. Cases running less than four weeks we have classified as acute. We realize this division is somewhat arbitrary, but we found that within four weeks the acute symptoms generally give way to the chronic. The chronic cases may take an exceedingly chronic course. One patient told us he had suffered from the disease for three years. Of course, we had to take the patients word for this statement. Other chronic cases had run for several months and even a year before they came to the hospital. Most of the cases, however, were acute.

SYMPTOMS

In the acute cases the patient first complains of chills; says he caught cold, develops a fever, sometimes low, sometimes running as high as 104. He complains of pain in the chest and of an almost constant cough, in most cases worse at night. Occasionally there is a loss of voice and some patients complain of night sweats. The sputum is much increased, is frothy, frequently blood stained and is mucilaginous and stringy. In most of our cases the cardinal symptoms were cough, increased and frothy sputum, fever and pain in the chest. Some only complained of cough. The quantity of sputum in some cases was enormous.

The chronic form results from several acute attacks or from neglected acute attacks, or possibly from a different strain of spirochete. In connection with the last statement, however, we wish to say, that as far as our experience goes, the microscope has shown no difference in size of the spirochete, or in spirals, or in any other way to indicate a different strain in chronic cases. The main symptoms in the chronic form are cough of a long standing and worse at night, much frothy sputum. The quantity of sputum is less than in the acute cases. There is usually no fever, on the contrary, a subnormal temperature is common. There may be blood in the sputum and often there is active hemorrhage. Physical examination shows signs very much like those in bronchitis, bronchial spirochetosis is a form of bronchitis, due to a specific cause. The findings are, however, by no means constant and are often hard to detect. Dry or moist râles may be heard usually over the larger bronchi and especially on inspiration. The pulse shows no peculiarity. It rises with the temperature. Respiration shows a slight increase as one would expect and is more rapid in the acute than in the chronic cases.

The general appearance of the patient is not so much like that of the tubercular sufferer with his stoop-shoulder, as that of a chronic bronchitis case. He is pale and the hemoglobin usually below normal.

Most of the cases treated at the hospital were acute.

PATHOLOGY

We have not been able to examine one case post-mortem.

SEASON

Although cases were found in every month of the year, the greatest number of cases were treated in the spring. Some patients reported that they had similar attacks previous years during the same season. We treated the greatest number of patients suffering from this disease during the months of April and May.

SEX AND AGE

Of the in-patients 39 were males and only 1 a female. Of the clinic patients a much higher per cent were women. From the first of January, 1922, to September 22, we treated in the clinic 7121 males and 1774 females, or about 20 per cent of all our patients were females. Of the total bronchial spirochete cases coming to the clinic, 13 per cent were women and 87 per cent were men. In all we treated in the clinic 166 cases (145 men and 21 women) as uncomplicated bronchial spirochete cases. In 97 other cases, not primarily bronchial spirochetosis, the spriochete was found in the sputum. As this paper is being finished, a foreigner, who came to China about one year ago, came to the hospital with definite bronchial spirochetosis symptoms and his sputum examination showed the spirochete. A few other foreigners were found to have the spirochete. From the above it seems as though men are slightly more liable to the infection than women, and this may in part due to the greater seclusion of the Chinese women.

As to age there is no doubt that the spirochete prefers the young. The oldest patient we treated was fifty-three years, the youngest only seven years. More than half the cases were between twenty and thirty and about 85 per cent were between twenty and forty years of age.

OCCUPATION

Evidently the occupation did not have very much to do with the disease, except that where people crowd together, as in schools, domitories, and barracks, the number is greater than in the open air occupations. Of the 40 in-patients 13 were soldiers, eight were students, and the others were divided among the following: cowherd, business man, coolie, housewife, farmer, peddler, richshaw coolie, officer Indian police, and servants. Except for soldiers and students practically all were natives of Riangsu and Ahnwhoi and near Nanking.

COMPLICATIONS

About half of our cases were uncomplicated. Of the clinic cases about two-thirds were reported as uncomplicated. The complications in the frequency of their occurrence were pneumonia, syphilis, tuberculosis, hookworm, malaria, and relapsing fever. The relapsing fever case came in for the fever, received the salvarsan treatment and cleared up of both diseases.

LABORATORY FINDINGS

All the cases from which we drew our information were diagnosed in our laboratory under the supervision of Miss Grace Bauer, foreign laboratory technician of the University Hospital. Owing to her careful work as well as to the interest of the foreign nurses in the hospital, this study has been made possible. In all cases the bronchial spirochete was definitely diagnosed and the gradual decrease of the spirochete recognized in most cases after treatment.

Blood examination on these cases show a high white cell count in the acute form. Dr. Wassel of Wuchang, lays special stress on high eosiniphilia. Our cases did not particularly show this.

STAINING

The following method used in the University Hospital laboratory is very simple: Fix sputum in flame. stain with gentian violet for three minutes, wash and dry with blotting paper. Examine under oil emersion and the spirochete shows up violet in the sputum. Fresh sputum unstained can be examined under high power for motility of the spirochete.

PROGNOSIS

From our experience we conclude, that the disease responds fairly well to treatment. All uncomplicated cases gave evidence of improvement very soon. Five cases left the hospital unimproved, and of these 2 were complicated with tuberculosis, 1 with pneumonia, and 1 left before a positive typhoid diagnosis could be made although the symptoms all pointed that way. The last left seven days after treatment was started, whereas his condition was of one and a half years standing. The average stay of the patients in the hospital was two weeks. Practically every patient of the uncomplicated cases left the hospital hopeful for a complete recovery.

TREATMENT

Following the suggestions of others, we used arsenic in some form, usually and with the greatest success in the form of Fowler's solution giving four drops three times a day. Salvarsan injections were also given and a quicker response was obtained, but most of the patients were unable to pay for them. For cases in which the sputum contained blood, we added to the treatment calcium lactate in five grain doses three times a day. Rest in bed, especially the first week, was insisted upon. For the cough some cough mixture was given, and if the pain in the chest was great aspirin was prescribed. Castellani suggests the following prescription for the cough:

Tartar emetic	gr. iii
Syrup tolu	℥ i
Aqua chloroform	ʒ iii

Sig. Teaspoonful in water every two hours

Our clinic patients were also put on arsenic and with a similar improvement. We added to the arsenic also our stock cough mixture.

In Castellani's last edition he recommends the use of tartar emetic combined with arsenic.

SUMARIO

Aunque es aparente que esta enfermedad no es muy prevalente en China, en Nanking y sus alrededores sin embargo, existen muchos casos. La causa directa es sin duda el espiroquete bronquial descrito ya por Castellani, Fantham y otros investigadores. El espiroquete presenta de cinco a ocho espirales y varía en longitud de 5 a 30 micrones por 0.2 a 0.6 micrones de ancho. Déspues de un periodo de motilidad adquiere una forma cocoidea de la que los nuevos espiroquetes se forman. Puede distinguirse de los Espiroquetes pallida, buccalis y dentium. Debe tenerse presente gue el *Sp. buccalis* puede encontrarse en todos los casos de espiroque tosis bronquial.

La infección se verifica por transmisión directa del espiroquete de persona a persona. La enfermedad debe caracterizarse como una bronquitis de causa especifica. Puede clasificarse como aguda o crónica. En los primeros hay tos, mucha flema espumosa y a veces sanguinolenta, fiebre y dolores en el pecho. Los casos crónicos presentan fiebre subnormal y a veces hemorragias. Son de larga duración.

El exámen microscópico se hace mediante el exámen de un frote de esputo, fijado a la flama y teñido durante tres minutos con violeta de genciana.

De enero a septiembre fueron observados un total de 7,121 varones y 1,774 mujeres, las dos terceras partes de los cuales no presentaban otras complicaciones. La mitad de los casos ocurrieron en personas de veinte a treinta años de edad. Las complicaciones existentes en muchos de los caos, cerca de una tercera parte, consistian en pulmonía, sífilis, tuberculosis, uncinariasis, malaria y fiebre recurrente.

SOCIETY NOTICES

ANNUAL MEETING

The American Society of Tropical Medicine will hold its regular annual meeting June 25 and 26, 1923 at San Francisco, California, where also during the same week the meeting of the American Medical Association is to be held. As the scientific sessions of the American Medical Association do not begin until Wednesday there will be no conflict in the programs of the two organizations, Monday and Tuesday being the meeting days of the Society of Tropical Medicine.

Members who desire to present papers should send titles at an early date to the Secretary so that the Program Committee can give them a suitable place on the program which it is expected will be printed and mailed to members on or about June 1.

MEMBERSHIP

On behalf of the Membership Committee attention is called to the fact that the Society has room for about 100 new active members without exceeding the limit of 300 established in the By-Laws. The continued welfare of the Society and particularly the permanency of its official JOURNAL is largely dependent upon the healthy growth and maintenance of the numerical strength of its active membership. Members are again urged to send to the Secretary the names and addresses of eligible persons desiring or likely to accept membership in the Society. This should be done soon so that the necessary data concerning the fitness and desirability of prospective members for membership may be secured in time for the consideration of the Council prior to the June meeting of the Society when the next election of members will be held. Under the By-Laws, any regular American physician or scientist interested in the study of tropical diseases is eligible to active membership. The Membership Committee would also like to receive for consideration proposals for Corresponding Membership. Foreign physicians or scientists who have contributed to the knowledge of tropical diseases are eligible to this class of membership. The dues of

Corresponding Members are $4.00 a year, including subscription to the AMERICAN JOURNAL OF TROPICAL MEDICINE. Corresponding Members thus receive not only the benefits of membership in the Society, but a Journal of value to everyone interested in tropical diseases, at less than the cost of the JOURNAL alone by separate subscription. In view of the particular interest of the Society in the Western Hemisphere it seems especially desirable that we should have on our list of Corresponding Members a greater number of representatives from Latin America than is the case at present. Their association with the Society cannot fail to be beneficial both to them and to other members with whom they are thus brought into relationship, through the increased opportunity afforded for communication with other workers in tropical medicine, a field in which individual isolation so far as concerns scientific interests is a common condition.

B. H. RANSOM,
Secretary-Treasurer.

BOOK REVIEWS

Aids to Tropical Hygiene. By Colonel R. J. Blackham, C.B., C.M.G., C.I.E., D.S.O., M.D., F.R.F.P.S., M.R.C.P.E., D.P.H. (Lond.). Second edition. Enlarge, thoroughly revised and mostly rewritten. Cloth. Price $1.75. Pp. 240. New York, William Wood & Company. 1922.

The purposes of this little volume are (1) to provide students, preparing for degrees in tropical hygiene, and officers of the Royal Army Medical Corps, going up for their promotion tests, with a ready means of reviewing their reading before examination; and (2) to furnish young practitioners a vade mecum containing a summary of the main facts with which health officers in the tropics must be acquainted. It deals with the climate of the tropics, the effects of tropical residence, acclimatization, air and ventilation, water and water-supplies, food and feeding, clothing in the tropics, tropical shelter—including sites, soils and houses, disposal of refuse in the tropics, disposal of the dead, the propagation of tropical diseases by insects, arachnids and rats, the relation of animal parasites to disease in the tropics, the prevention of malaria, and disinfectants and disinfection. The text is followed by an epidemiological table of diseases in which the modes of infection, incubation periods, methods of isolation, handling of contacts, and preventive methods are arranged in ready-reference form, covering approximately 40 transmissible diseases. An appendix contains a discussion of meteorology, including those phenomena—temperature, atmospheric humidity, rainfall, atmospheric pressure and winds—which concern the tropical hygienist.

The book teems with concise and interesting statements of tropical conditions as they actually exist, which are reminiscent to one who has experienced tropical exile and which immediately stamp the author as an authority who writes from an experience that has been both practical and broad. Although the essential value of the work is its practicality such scientific and theoretical conceptions as are treated of are presented, with very few exceptions, in an admirably up-to-date way. The ground that is covered in this small volume is really remarkable; but, as is inevitable in works of this sort, certain omissions have occurred. A serious omission from the point of view of the American reader is that hook-worm disease and yellow fever are not discussed. The value of the book would be enhanced, also, by by the addition of concise clinical data in regard to prodromata and early signs of the transmissible diseases discussed, as an aid to that most important preventive measure —early diagnosis of the first case or cases. Altogether, however, in a style that makes it most interesting reading, Colonel Blackham has made a book that not only fulfills its purpose, but which meets a distinct need in America. It should be welcomed by medical officers of the public services, public health workers, students of tropical medicine, and practitioners in sub-tropical and tropical regions in general.

George B. Foster, Jr.
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The Diagnostics and Treatment of Tropical Diseases. By E. R. Stitt, Fourth edition, revised, with 159 illustrations. Philadelphia, P. Blakiston's Son Co.

The remarkable success of the author's "Practical Bacteriology, Blood Work and Animal Parasitology" and of the present work makes it interesting to review their genesis. In the preface of the first edition of this book, we read that, while in general medicine, the diagnosis is made by a close correlation of bedside and laboratory findings, in tropical medicine, the diagnosis has largely been made in the laboratory. It was the author's original intention to treat tropical diseases in the same way as other diseases, namely, by making the diagnosis depend on all information available whether from bedside or laboratory: but he was forced to abandon this plan because of the lack of clinical experience of the average student. "There was not the same difficulty attaching to a book exclusively devoted to the diagnostic methods of the laboratory so that in 1908 a laboratory manual was published." Practical Bacteriology may therefore be considered as a contribution of tropical medicine to general medicine. That it is a substantial contribution is evident from the fact that it is now in its 6th Edition. A more intimate evidence of its popularity and value is seen in the worn condition of the copies in most clinical laboratories. It is the favorite "hand" book for technicians and physicians.

With the increase in clinical knowledge of tropical diseases in recent years, the author went back to his original plan and in 1914 wrote a general book on tropical diseases which is now in its 4th Edition. Although it is therefore a companion volume to the first one, it is complete in itself. Only a teacher with rare discrimination and patience could compress so much valuable and accurate detail in so small a compass.

The general feature of previous editions are maintained. The author starts with a consideration of each disease and gives definition and synonyms, history and geographical distribution, etiology and epidemiology, pathology, symptoms in general and in detail, diagnosis, prognosis, prophylaxis and treatment. The diseases are grouped as protozoal, bacterial, deficiency, helminthic, granulomatous, skin diseases and those of disputed nature or minor importance.

The distinctive second part deals with the diagnostics of tropical diseases in general and contains unique chapters which cross reference and supplement the first part. These chapters deal with diagnostic problems and procedures, onset and temperature chart; blood examinations; circulatory; respiratory and lymphatic systems; jaundice and the liver and spleen; the cutaneous system and organs of special senses; the urine and genito-urinary apparatus; the feces and alimentary tract; the joints, bones and muscles; neurological considerations. This arrangement makes it rather difficult at first to gather up all the threads but increasing familiarity with the plan of arrangement shows the value to the student of presenting the subject "from every angle."

The new material includes the acceptance of the spirochetal etiology of yellow fever, advances in food deficiency diseases, beriberi and pellagra, the administration of arsphenamine and antimony, new chapters on epidemic jaundice, rat bite fever, tularaemia, and the findings in blood chemistry. As a small manual, it stands unique and self-sufficient among the more bulky works on tropical medicine.

While recognizing that, on final analysis, any work of this kind depends on the initiative and perseverance of the individual author, it is a pleasure to note that, in a sense, this book is the product of the Naval Medical Corps and Naval Medical School and that the author now holds the highest position within the gift of the Navy Medical Department. The Government Services give an opportunity for scientific work, but, of course, they do not insure it. Given the opportunity, everything else depends on the individual and we recall that Sternberg published his pioneer and monumental "Manual of Bacteriology" while Medical Supply Officer in San Francisco. The most important work of the Services is undoubtedly administrative or the maintenance of the organization, and it is only natural that most medical officers should be primarily administrators, but it is encouraging to know that, when outstanding scientific workers like Sternberg and Stitt do appear, they are rewarded by their organizations. In this way they are able to introduce and insist on specific scientific methods and to support definite investigations which in the long run always yield practical results for the Services.

H. J. Nichols.

Précis de Parasitologie. Brumpt (E.). Troisième Édition. Entièrement remanieé. Pp. xv + 1216. With 736 figures and 5 plates. 1922. Paris: Masson et Cie., 120, Boulevard Saint-Germain.

The second edition of this work appeared in 1913 and was exhausted five years ago. For this delay in the appearance of the third edition, Professor Brumpt pleads the pressure of other important labors and, too, the necessity of devoting long hours to the analysis, evaluation, and resumé of the many important contributions to the science of Parasitology that have appeared in the past ten years. And it is very obvious that the author has, in fact, diligently collected, patiently considered and judiciously chosen a large amount of new material for the thorough revision of his Precis.

The general plan of this third edition is the same as that of the second. After a chapter on General Principles of Parasitology (46 pages), the pathogenic Protozoa are discused (264 pages) under the broad headings of Spirochetes (44 pages), Rhizopods (30 pages), Sporozoa (80 pages), Flagellates (97 pages) and Infusoria (13 pages). It will be noted that the Spirochetes are not considered with the Flagellates as in the second edition but are given a separate and equivalent place.

The second section deals with Medical Helminthology (352 pages): Trematodes (83 pages), Cestodes (91 pages) and Nematodes (178 pages).

The Arthropod Parasites are treated next (231 pages) under the principal titles of Arachnids (78 pages), Hemiptera (30 pages) and Diptera (123 pages).

Finally, there is presented the subject of medical Mycology (229 pages) the Hyphomycetes occupying 159 pages, the Ascomycetes 43 pages and the Phycomycetes 10 pages. In this section, it is noted that Professor Brumpt has adopted the classification of Vuillemin and there is accordingly a rearrangement of the text matter. Thus the genera Trichophyton, Epidermophyton and Achorion have been taken from the group of Ascomycetes and placed with the Hyphomycetes. In the text proper, however, there are few changes or additions.

Bacteriology is not considered for the reason that the book is intended particularly for medical men in the tropics and because there are many texts that deal adequately with cosmopolitan bacterial diseases.

The important parasites are given special treatment under the following general plan: Synonymy, Description, Habitat, Evolution (Life History), Transmission (Vectors) and Pathogenesis.

Diseases caused by the various parasites are briefly described either in connection with the causative virus, e.g., Chagas' Disease and *Trypanosoma cruzi;* or with the responsible helminth, e.g., Filariasis; or in case the virus is insufficiently or totally unknown, in connection with the transmitting agent, e.g. Typhus and Trench Fever with Pediculus and Rickettsia bodies, Dengue Fever and Stegomyia, etc. Even certain bacterial diseases require particular though brief treatment: Plague and the Flea, Tularemia and Chrysops. But it is to be noted that, in general, clinical descriptions are very subordinate to considerations of the biology of the parasites concerned.

Technical methods for diagnosis and general laboratory work in Parasitology are not treated.

New material incorporated in this edition reminds us of the great progress made during the past ten years. Thus the role of the Leptospira group of the Spirochetes in Infectious Jaundice, Rat-bite Fever, Seven-day Fever and Yellow Fever is adequately discussed. We may note here that the author accepts Leptospira as a synonym only for certain of the Treponemata. American Relapsing Fever caused by *T. venezuelenze* and transmitted by *Ornithodorus venezuelensis* and *O. talaje* is also new. Human coccidiosis has grown in importance, Bartonella and Oroya Fever is given place for the first time. American Leishmaniasis (Forest Yaws) and American Trypanosomiasis (Chagas' Disease) are much enlarged. These two diseases and their viruses are excellently discussed and with an authoritativeness due, perhaps, to the author's long sojourn in Brazil on the Faculty of Medicine at Sao Paulo. As new material we may mention further, in the section on parasitic worms, that the following life cycles have been cleared up: *Fasciolopsis buski, Clonorchis sinensis, Paragonimus westermanni, Metagonimus*, the three Schistosomes, *S. japonicum, S. mansoni, S. hematobium,* and *Dibothriocephalus latus;* while an account of the migrations of the Ascarids and Trichocephalus (=Trichuris) is also presented. In the section on the Arthropod Parasites the author discusses for the first time the various Rickettsia viruses and the role of Chrysops in the transmission of Tularemia (Deerfly Fever).

The Precis de Parasitologie has 736 figures in the text of which 302 are original. On the whole they are well done. An exception, e.g., is Figure 21 of *T. pertenue* which can not be reconciled with the text. Of the colored plates, two are the well-known plates of the Microfilariae from Manson's Tropical Diseases, while the other two illustrating the malarial parasites are only fair. But in general the illustrations, very profuse, are good and excellently chosen to serve the prime purpose of aiding the text in the exposition of the subject. Very effective use, too, is made of tables summarizing certain features such as developmental and life cycles, taxonomic groups, generic and specific differences, vectors of disease, etc.

The author has omitted from this edition the greater part of his bibliographical references. It is of interest to note that he states in his preface that the text would have been clearer if he could have omitted completely the mention of names of authors to whom we owe our actual knowledge. But a text on a biological science in the course of development cannot be treated thus. Accordingly he does cite publications that will serve as a starting point in the bibliography and very generously mentions numerous authors, as far as possible in chronological order.

The book covers a very large field and contains an immense amount of facts. Yet the material is presented in a strikingly orderly fashion due to careful arrangement, the use of titles and sub-titles for subjects and paragraphs, the employment of a varety of printer's type of different sizes, and above all, a thorough knowledge of the subject on the part of the author that makes possible a perspective and proper appreciation of the relative values of innumerable particulars. The text is a model of exposition, simple, direct, clear.

The Precis de Parasitologie is something more than a "summary," especially as regards the parasites themselves. It is good to have a presentation of the subject from one of the French school and particularly by Professor Brumpt whose extensive first-hand knowledge of Parasitology manifests itself in a distinct individuality of method and independence of other texts dealing with the same subject.

This text is one of a "Collection de Precis Medicaux" from the press of Masson et Cie., is inexpensive, and suitably printed and bound.

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HOOKWORM DISEASE AS RELATED TO INDUSTRY IN AUSTRALIA[1]

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In mines, hookworm disease is well recognized as an occupational disease. Long before the cause was known, it was observed that the "miners' anemia" of Central and Northern Europe was unmistakably related to the occupation of mining, for people who did not go underground were not affected.

Even above ground hookworm disease may be an occupational disease in parts of the tropics and subtropics, especially among agricultural workers. After measuring the degree of infection of many people in Brazil by determining the number of hookworms expelled by treatment, Smillie[2] has expressed the opinion that in the tropics and subtropics "hookworm disease is an occupational disease and is largely limited to soil workers."

There are occupations other than mining and tilling the soil which have at times been found to favor the spread of hookworm infection even in regions where surface conditions would normally be unfavorable to the spread of infection, such, for example, as tunneling and brickmaking. But we have no evidence that these two occupations have been responsible for hookworm disease in Australia, where the observations presented in this paper were made. We shall, therefore, consider mining and tilling the soil, since these are the kinds of work most likely to favor the spread of hookworm disease in Australia and her island territories.

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[1] Read before the second annual meeting of the Public Health Association of Australasia, Sydney, September 12 to 16, 1922. The Australian Hookworm Campaign, on the work of which this paper is largely based, is conducted with the support and under the auspices of the Commonwealth, states, and territories of Australia, and the International Health Board of the Rockefeller Foundation.

[2] Smillie, W. G., Studies on hookworm infection in Brazil, 1918–1920, Second Paper, Monographs of the Rockefeller Institute for Medical Research, No. 17, May 12, 1922, p. 30.

INVESTIGATION OF AUSTRALIAN MINES

In four states in Australia, South Australia, Queensland, New South Wales, and Tasmania, but not in Western Australia or Victoria, ancylostomiasis is a compensable disease, under Workers' Compensation Acts if contracted as the result of the occupation of mining. This recognition of hookworm disease as an industrial disease was apparently due solely to European experience and precedent and was intended as a precaution for the future, for when these acts were passed there was no definite evidence that any of the Australian mines were infested.

Representative mines have now been examined by the Australian Hookworm Campaign in all the states and in Papua. No hookworm infection was found in the coal or metalliferous mines investigated in Victoria, Tasmania, and Western Australia. In New South Wales, in over 1200 examinations of miners of the Newcastle-Maitland coal-fields, only 5 infected miners were found, 2 of whom had probably contracted their infection outside this region. Under the Technical Commission of Inquiry 3788 miners in the metalliferous mines at Broken Hill in New South Wales were examined for hookworm disease by a staff loaned from the Australian Hookworm Campaign. No hookworm disease was found.[3] In Queensland no definitely infested metalliferous mine has yet been discovered, but hookworm disease was found to be prevalent and related to the occupation of mining in the Ipswich and Burrum groups of coal-mines. In Papua considerable hookworm disease was found among native miners, but the occupation of mining had apparently not, as yet, increased the amount of infection above that of the natives of the same region who did not engage in mining.

[3] Report of the Technical Commission of Inquiry to Investigate the Prevalence of Miners' Phthisis and Pneumoconiosis in the Metalliferous Mines at Broken Hill, Department of Labour and Industry, New South Wales, 1921, Chapter VII, pp. 44 to 57.

TABLE 1

Examinations of miners in Australia and Papua for hookworm disease, up to September 1, 1922

	TYPE OF MINE	APPROXIMATE NUMBER OF MINES EXAMINED	NUMBER OF MINERS EXAMINED*	INFECTED WITH HOOKWORMS	PERCENTAGE INFECTED
South Australia:					
Wallaroo and Kadina	Copper	2	228	0	0
Moonta	Copper	1	26	0	0
New South Wales:					
Broken Hill group	Lead, silver, zinc	10	3,788	0	0
Newcastle-Maitland group	Coal	23	1,224	5	0.41
Queensland:					
Ipswich group	Coal	26	1,030	324	31.5
Burrum group	Coal	7	264	208	78.8
Mt. Morgan	Gold, copper silver	1	164	2	1.2
Baralaba	Coal	1	101	1	1.0
Blair Athol	Coal	1	90	0	0
Gympie	Gold	1	117	0	0
Western Australia:					
Kalgoorlie	Gold	2	140	0	0
Collie	Coal	1	15	0	0
Victoria					
Bendigo group	Gold	36	632	0	0
Wonthaggi	Coal	1	374	0	0
Northern Territory					
Marranboy	Tin	1	10†	0	0
Tasmania:					
Mt. Nicholas and Cornwall	Coal	2	18	0	0
Queenstown and Gormanston	Copper, silver	2	44	0	0
Derby, Branxholm, and Weldborough	Tin	3	30	0	0
Zeehan	Silver, lead	1	16	0	0
Waratah	Copper, silver	1	18	0	0
Papua:					
Dubuna‡	Copper	1	61	28	45.9
Laloki‡	Copper	1	84	36	42.9
Misima Block 10‡	Gold	1	434	316	72.8
Total		126	8,908	920	10.3

* In this column "miners" often include mine employees working on the surface.

† Estimated.

‡ Native labor.

In table 1 are shown the results of the examinations of Australian mines up to September 1, 1922. An investigation of the Cloncurry (Queensland) group of copper, silver and gold-mines were attempted but most of them were closed at the time. Nevertheless, 1299 persons including many former miners, were examined in this region, and no locally contracted cases of hookworm disease were found. Examinations of moderate numbers of people including miners were also made at Charters Towers (gold mining) the Bowen coal-mining field, the Kangaroo Hills tin fields, and the Styx coal mines, all in Queensland, and the general conclusion was that hookworm disease was not then prevalent in any of these mines. In North Queensland and New South Wales several important mines remain to be studied.

In all these investigations the examinations of miners for hookworm infection were made by preparing specimens of their feces, using either the centrifuge method or salt-flotation, and then examining for ova under the microscope.

All of the mines in table 1 shown to be free from hookworm disease are in regions where there is no infection, or very little, among people working on the surface. Of the infested mines, the Ipswich coal-mines are outside the belt in which hookworm disease can be regarded as endemic among those who stay on the surface, for the examination of 1698 school children in this area revealed only 10 infections, giving an infection rate of 0.69 per cent. The infested coal-mines of the Burrum group are in a coastal belt of high rainfall, and the people engaged in pursuits other than mining are infected to a considerable extent, but not nearly so much as the miners, as is shown in table 2.

Among the many mines examined, the only groups which have been definitely shown to be spreading hookworm disease are the Ipswich and Burrum groups of coal-mines in Queensland. The severity of the infection in these two mine groups cannot be judged from records of absences from work, as, unfortunately, the books kept at the mines do not discriminate between absences due to sickness and the much larger number due to other causes. It is important for the welfare of the industry and the miners that more detailed and complete records should be kept in such mines and be available for studies of industrial diseases.

TABLE 2

Hookworm infection in the Burrum coal area, by occupations

	NUMBER		ADULTS IN OCCUPATION	ADULT MEMBERS OF HOUSEHOLD OTHER THAN MINERS	SCHOOL CHILDREN SIX TO FOURTEEN YEARS	INFANTS TO FIVE YEARS
Miners, infected	186	Examined		144	204	83
		Infected		38	117	15
		Percentage		26.4	57.4	18.0
Miners, non-infected	38	Examined		40	38	13
		Infected		4	14	1
		Percentage		10.0	36.8	7.7
Total miners (underground)	224	Examined	224	184	242	96
		Infected	186	42	131	16
		Percentage	83.0	22.8	54.1	16.7
Mine employees, infected	22	Examined		32	32	14
		Infected		5	16	0
		Percentage		15.6	50.0	0
Mine employees, non-infected	18	Examined		24	15	9
		Infected		3	6	1
		Percentage		12.5	40.0	11.1
Total mine employees (surface)	40	Examined	40	56	47	23
		Infected	22	8	22	1
		Percentage	55.0	14.3	46.8	4.3
Townspeople, infected	13	Examined		20	13	3
		Infected		6	10	1
		Percentage		30.0	76.9	33.3
Townspeople, non-infected	42	Examined		65	33	32
		Infected		8	6	1
		Percentage		12.3	18.2	3.1
Total townspeople	55	Examined	55	85	46	35
		Infected	13	14	16	2
		Percentage	23.6	16.5	34.8	5.7
Farming people, infected	14	Examined		15	26	7
		Infected		6	18	0
		Percentage		40.0	69.2	0

TABLE 2—*Continued*

	NUMBER		ADULTS IN OCCUPATION	ADULT MEMBER OF HOUSEHOLD OTHER THAN MINERS	SCHOOL CHILDREN SIX TO FOURTEEN YEARS	INFANTS TO FIVE YEARS
Farming people, non-infected....	15	Examined		27	11	4
		Infected		2	3	0
		Percentage		7.4	27.3	0
Total farming people...........	29	Examined	29	42	37	11
		Infected	14	8	21	0
		Percentage	48.3	19.0	56.8	0

One criterion by which the effects of hookworm disease in these mines might be judged is the amount of anemia as shown roughly by the hemoglobin percentage according to the Tallqvist hemoglobin scale. The examination of 13 non-infected miners of the Ipswich group gave hemoglobin percentages ranging from 80 to 95 per cent and averaging 87.1 per cent. The corresponding figures for 12 infected miners were 70 to 85 per cent with an average of 80.4 per cent. The difference between these two average percentages, or approximately 7 per cent of the normal, shows a definite reduction in the amount of hemoglobin, presumably largely owing to hookworm disease. None of the cases showed pronounced symptoms and all were able to work. In the Burrum coal-fields the blood of 115 miners was tested by Drs. C. N. Leach and C. P. Rosenthal of the Hookworm Campaign, and the hemoglobin percentages were found to range from 60 to 100 per cent, and the average was 77.5. In this series, miners were examined as they came and non-infected men were included. Seven men had percentages of 60 or 65.

Another measure of the severity of the infection is the determination of the number and species of hookworms present, for experience shows that the larger the average number of worms harbored and the higher the proportion of hookworms of the species *Ancylostoma duodenale* to those of the species *Necator americanus*, the greater is the injury to health. As the infected miners could not spare three consecutive days from work for complete worm counts, the number of counts made was small and most of them were incomplete. One man who had been a miner in the Burrum coal-fields seven years before was examined in the hospital at Rockhampton, a non-infested area. In three days following the administration of oil of chenopodium he expelled 241 hookworms, all of the species *Necator americanus*. Most of these may have been acquired outside the mines, although the medical officer investigating the case considered the mines the most probable source. At the Ipswich coal-mines it was attempted to make counts at the miners' homes during week-ends, and 11 partial counts were made after the small routine dose of 1.5 cc. cf oil of chenopodium. In 7 cases the stools were saved for only twenty-four hours, and in the other 4 for only two days. In 2 of the one-day counts no worms were recovered. In the other 9 counts, 82 worms of the species *Necator americanus* were secured, an average of 9 worms from each individual, probably only a small part of the total number harbored. The evidence from the percentage of miners infected, hemoglobin percentages, and worm counts suggests that the infection is more severe in the Burrum mines than at Ipswich, that the species *Necator americanus* predominates, and that the infection is doing damage to health, although it seldom causes complete incapacity for work.

It is not entirely clear why hookworm disease has not become prevalent in certain mines, for example, the Newcastle-Maitland coal-mines of New South Wales, in which the conditions of temperature, moisture, pollution of the earth, and occasional introduction of infected miners are favorable to the spread of hookworm disease. It is suspected that one of the important factors tending to inhibit the development of hookworm larvae in mine earth is a high content of dissolved mineral salts in the mine waters. In the series of analyses available, acidity was slight or absent in nearly all cases and not sufficient to be an important factor in preventing the development of hookworm larvae.

In the Great Boulder Mine at Kalgoorlie, Western Australia, according to an analysis on record at the mine, the mine water contains over 120,000 parts of solids per million and several times as much sodium chloride as is found in sea water. This highly mineralized mine water would with certainty inhibit the development of larvae.

In connection with the investigation of the Commission of Inquiry into miners' diseases at Broken Hill, an attempt was made to ascertain whether the salts in the waters of the local mines would inhibit the development of hookworm larvae, as the ten deep mines at Broken Hill were entirely free from hookworm disease.[3] Contrary to expectations thirteen highly mineralized mine waters permitted the development of hookworm larvae in cultures made up of charcoal and feces containing ova, and kept saturated with the water tested. One of the mine waters tested in this way contained 3.4 per cent of total solids. It completely inhibited the development of the larvae when concentrated to double strength by boiling. In this mine water there was included among the salts present a considerable quantity of zinc sulphate. To test further the effect of this salt, cultures were wet with a 4 per cent solution of zinc sulphate. Larvae developed in the charcoal cultures, but over 3.5 per cent of zinc sulphate inhibited development when sand was used instead of charcoal in making the cultures. Development was inhibited completely in cultures wet with 2 per cent sodium chloride solution but larvae were present in large numbers when only 1.5 per cent sodium chloride solution was used. When mine earths were substituted for charcoal in the cultures used in testing the mine waters, the larvae were much fewer in number and sometimes absent.

Twelve mine-water samples from the Newcastle-Maitland coal-fields were examined for the Hookworm Campaign by the New South Wales Department of Public Health, through the courtesy of the Director-General of Public Health. With one exception, the 12 samples had a high content of mineral salts, and in most instances sodium chloride was the predominant salt. If 2 specimens not typical of this group of mines are excluded, the specimens range in their content of total solids from 2187 to 20,620 parts per million, and in content of sodium chloride from 1041 to 17,140 parts per million. All but 3 of these samples were alkaline. One of the excluded samples was highly acid.

Six samples of mine waters from the infested mines were analyzed—3 from the Ipswich fields and 3 from the Burrum fields. They ranged in total solids from 470 parts per million to 6943 parts. In sodium chloride content they ranged from 325 to 3003 parts. None of the 6 waters from infested mines had more than 0.7 per cent of total solids and 0.3 per cent of sodium chloride, while eleven out of twenty-one of the waters from non-infested mines exceeded one or both of these amounts. The evidence fails to show that the salt content or reaction of the non-infested mines is alone responsible for the absence of hookworm infestation, as a considerable number of these mines have waters with relatively low amounts of total solids and sodium chloride, and low acidity or alkalinity.[4]

It seems possible that in many cases the dissolved mineral salts, although insufficient in amount to prevent completely the development of hookworm larvae in laboratory cultures, may have a slight deleterious influence which would favor the gradual disappearance of an infestation, thus tending to keep certain of the mines free from hookworm disease. It seems probable that several factors other than the contents of the mine waters enter into the problem and will be necessary to the explanation, except in those few cases where the mine waters are highly above the average in salinity or very acid.

CONTROL MEASURES IN MINES

The control of hookworm disease in Australian mines would not be difficult. The total number of underground workers in the two infested groups of coal-mines is only about 1300, and most of these are in the more lightly infested of the two groups. At the beginning of the investigation nearly all the infested coal-mines, and some of the non-infested ones, were without underground latrines, and the men defecated in the unused workings. In metalliferous mines underground latrines are usually found and there is provision for taking the full pans to the surface. There is a prevalent belief, however, that such care is not necessary in coal-mines, and this is reflected in the policies of the State Mines Departments. In Queensland a number of new underground panstead cabinets with lids have recently been installed in the Burrum Shire group of mines. The Queensland State Department of Mines has sent letters to the operators of coal-mines calling attention to the need for one latrine pan and seat with a supply of deodorant for every 20 men working underground in the infested coal-mines. The Department recommends that the pans should be on a cement flooring and should be protected from overhead drip. The seats should be cleaned at least once a week and the pans should be covered with a tight cover and taken to the surface for emptying not less often than once in two days. The recommendations state also that provision should be made to prevent the miners from working underground with bare feet or leaky boots. This last advice is highly important for many of the coal-miners in the infested group worked barefoot and barebacked, as shown in figure 1. The bare back is probably not nearly so dangerous as the bare feet as most of the infection would come from the floors of the workings.

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[4] The analyses are given in full in Survey Report No. 19 of the Australian Hookworm Campaign.

In addition to circularizing the mine operators the Queensland Department of Mines has printed and distributed a cloth placard for the information of miners. The Australian Hookworm Campaign has treated all the miners found to be infected, but it is inevitable that a considerable proportion will be reinfected and will require further treatment before the preventive measures are fully effective.

TILLING THE SOIL

The statement of Smillie, previously quoted, that hookworm disease is an occupational disease largely limited to soil workers was based in part on four sets of investigations of the numbers of worms harbored by people in rural Brazil and the percentages of hemoglobin in their blood. His data were presented in four tables which have been condensed into table 3 for purpose of comparison.

The figures given in table 3 clearly support Smillie's contentions, as far as the areas investigated are concerned, for the field workers harbored on the average ten times as many hookworms as the others.

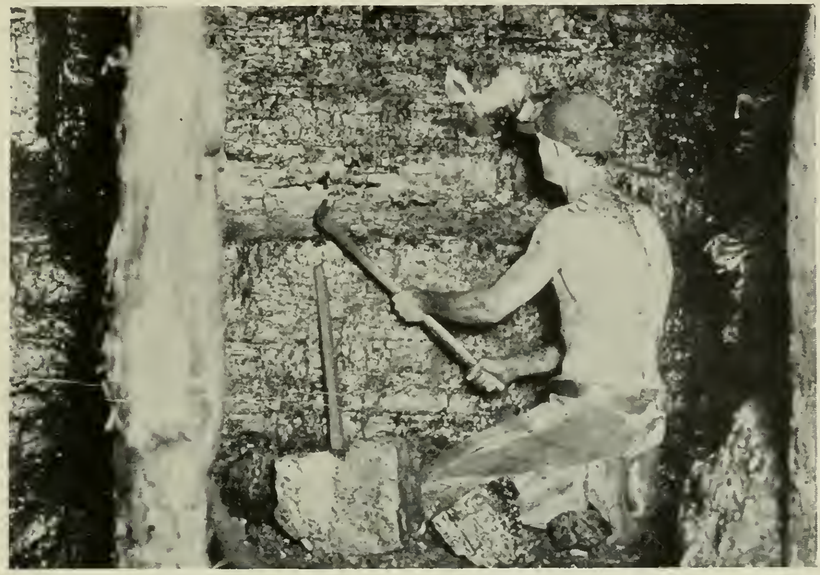


FIG. 1. MINER WORKING BAREFOOT AND STRIPPED TO THE WAIST IN THE BURRUM COAL MINES

TABLE 3

Results of investigations by Smillie of severity of hookworm disease in rural districts of Brazil

	NUMBER PEOPLE EXAMINED	AVERAGE AGE	AVERAGE HEMOGLOBIN PERCENTAGE	AVERAGE NUMBER OF HOOKWORMS
		years		
Adult field workers	171	26	63	273
Men doing other work	26	39	72	32
House workers	84	30	69	29
Children working in fields	60	12	56	234
Children not working in fields	66	9	65	20

In the regions studied in Brazil, according to Dr. Smillie's report, children commonly commence working in the fields at the age of eight, the girls beginning a little later than the boys. Nearly all of the field laborers work barefoot, and for long hours. They have no latrines at their homes. In Australia, on the other hand, in the warmer rural districts, most children and a few adults go barefoot by preference as well as for economy, but most of the adults wear shoes even when working. Children are required to go to school until they are fourteen years of age. Moreover, practically every home has a latrine of some sort. Under these conditions, in the white population of rural districts and small towns in infected parts of Queensland, the ratio of the rate of infection in school children (mostly barefoot) to that of adults (mostly wearing boots) was found to be on the average approximately 100 to 57.

In table 4 is shown the prevalence of hookworm disease among white people (almost the whole population), grouped by occupation, in several rural districts in Queensland and New South Wales.

From table 4 the conclusion can be drawn. that hookworm disease in the white population of Australia is about 50 per cent more prevalent among children of school age than among agricultural workers, and that it cannot in general be classed as an industrial disease related to tilling the soil. There is abundant evidence, however, in the records of the Hookworm Campaign to show that hookworm disease is a rural disease, even if not usually an occupational disease related to farming, and is relatively infrequent in the cities and larger towns. The most important single factor, however, in the localization of hookworm disease in the Australian tropics and subtropics has been the variations in the amount of rainfall, for hookworm disease practically disappears from the Australian white population where the average annual rainfall is less than 40 inches.

The infection rates in table 4 are for large areas and the farming groups include some kinds of agricultural work requiring relatively little intensive cultivation of the soil. In table 5 are given similar data collected by Dr. C. L. Paine, of the staff of

TABLE 4

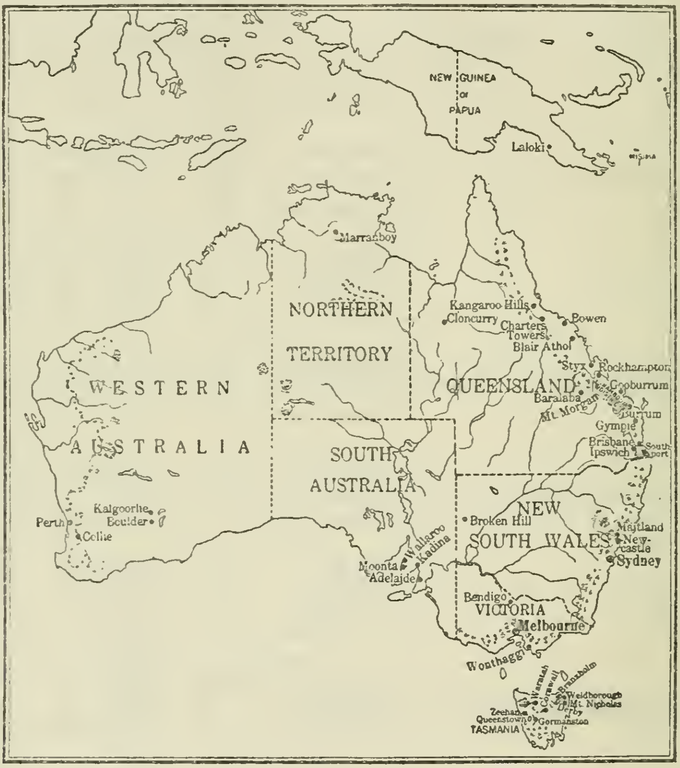
Hookworm disease in rural Australia, by occupations

		BOWEN DISTRICT,* QUEENSLAND	SOUTHPORT DISTRICT, QUEENSLAND	NAMBOUR DISTRICT, QUEENSLAND	NORTHERN RIVERS, NEW SOUTH WALES	TOTAL
School children (six to fourteen years)	Examined	1,116	2,024	3,105	1,234	7,479
	Infected	136	103	163	68	470
	Percentage	12.2	5.1	5.2	5.5	6.3
Infants (one to five years)	Examined	574	901	1,484	545	3,504
	Infected	26	16	33	11	86
	Percentage	4.5	1.8	2.2	2.0	2.5
Domestic duties	Examined	1,313	2,364	3,445	1,423	8,545
	Infected	73	47	61	28	209
	Percentage	5.6	2.0	1.8	2.0	2.4
Farmers and laborers	Examined	994	1,184	2,543	712	5,433
	Infected	102	47	61	20	230
	Percentage	10.3	4.0	2.4	2.8	4.2
Business and professional	Examined	267	357	337	581	1,542
	Infected	5	7	1	13	26
	Percentage	1.9	2.0	0.30	2.2	1.7
Skilled trades	Examined	168	280	293	248	989
	Infected	6	1	5	10	22
	Percentage	3.6	0.36	1.7	4.0	2.2
Miscellaneous and unclassified	Examined	238	390	1,406	130	2,164
	Infected	9	18	30	6	63
	Percentage	3.8	4.6	2.1	4.6	3.0
Total	Examined	4,670	7,500	12,613	4,873	29,656
	Infected	357	239	354	156	1,106
	Percentage	7.6	3.2	2.8	3.2	3.7

* The Districts referred to in this table are large areas usually named after a central town in which the Field Unit of the Hookworm Campaign had its headquarters.

the Australian Hookworm Campaign, for a small area in which sugar-cane is raised.

In table 5 it will be noticed that children (including infants) and wage-earning adults in a sugar-raising community in Queensland were about equally infected, but that those engaged in domestic duties were less infected. If the infants (one to five years of age) had been classified separately, the children would undoubtedly have shown a higher percentage of infection than the agricultural workers as a group. In table 2 data were given for a community in which agriculture and mining were going on side by side. From this table it will be seen that the children of farmers have a higher infection rate than the farmers themselves, the usual experience in Queensland. The children of



miners, on the other hand, had a lower infection rate than the miners working in infested mines. The children of the mining and agricultural groups were about equally infected. This suggests that, as a general rule, the children in Queensland get most of their infection near the home and at other places where they congregate to play barefoot, while the farming parent loses

TABLE 5

Hookworm infection in Gooburrum Shire, Queensland, by occupations

GROUP	WAGE-EARNING ADULTS			OTHER ADULTS IN HOUSEHOLDS			SCHOOL CHILDREN AND INFANTS			TOTAL		
	Examined	Infected	Percentage	Examined	Infected	Percentage	Examined	Infected	Percentage	Examined	Infected	Percentage
Farm group:												
Farmers and farm laborers	163	3*	1.9	119	3	2.5	188	5‡	2.7	470	11	2.3
Cane-cutters	71	6	8.5	34	0	0	62	3§	4.8	167	9	5.4
Total for group	234	9	3.8	153	3	2.0	250	8	3.2	637	20	3.1
Mill employees:												
Total for group	110	3	2.7	59	0	0	119	3	2.5	288	6	2.1
Town group:												
Total for group	33	1	3.0	25	1	4.0	36	0	0	94	2	2.1
Floating population:												
General	33	0	0	10	0	0	2	0	0	45	0	0
Field workers (cane, etc.)	55	0	0	0			0			55	0	0
Total for group	88	0	0	10	0	0	2	0	0	100	0	0
Miscellaneous:												
Total for group	8	0	0	3	0	0	2	0	0	13	0	0
Total	473	13	2.7	250	4†	1.6	409	11	2.7	1,132	28	2.5

* Including one boy at work, age not specified.

† All four were housemaids from thirteen to seventeen years of age, and three had come from farms.

‡ Three were children of a non-infected farmer, one was the child of another non-infected farmer, and one of an infected farmer.

§ All three in the family of a cane-cutter who was not infected.

more worms than he acquires, partly because he wears boots much of the time and partly because the fields are less polluted than the closer vicinity of the rural home. The same table shows that the children and adults of the small towns are less infected than those of the country. This is most probably due to the somewhat lessened soil pollution in the town and the greater inclination to wear shoes for the sake of appearance. Boys have been noticed to have more infection than girls, for the older girls are more inclined to wear shoes than their brothers of the same age.

In making these comparisons infection rates have been used, for, where the infection rate is low and based on large numbers of examinations, it is more reliable and more sensitive to moderate influences affecting the transmission of hookworm disease than an index based on the average number of hookworms harbored by the infected individuals. In the mines of the Burrum fields, where the infection rate was high, a knowledge of the average number of hookworms would have been valuable and further attempts are being made to secure this information. In most of the infested areas in Australia, however, less than one-fourth of the people are infected and counts of the hookworms harbored by patients selected on account of moderate pallor or an unusual number of ova, in the stools, or for other reasons, usually show few worms, although rarely a count of several hundred is obtained. Under these circumstances the worm index for an area (average number of hookworms harbored) is difficult to determine, and is of little value for purposes of comparison even if the number of people having no bookworms is considered in computing the index. For these reasons control measures, in the infested areas of Australia, including the prevention of soil pollution and the treatment of infected persons, are being determined and guided by a hookworm index, which is the infection rate of the whole white population of each area. The rates of special groups, such as school children, are corrected for age by means of a simple table.[5] Where thorough control is practicable, such an index is valuable, as it is highly sensitive to reinfections and can be permanently lowered only by keeping down new infections. On the other hand, where such control is impracticable the highest degree of relief of a heavily infected population would probably be aided best by watching closely the variations in the average numbers of worms harbored.

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[5] Sawyer, W. A., Some directions in which advances in preventive medicine could be made in Queensland, Reprint from Medical Journal of Australia. February 11, 1922, vol. 1, 9th year, pp. 141–144.

The need for the treatment of highly infected population groups, such as the agricultural groups considered in this paper, has been questioned by some workers in more highly infested countries. In Australia the evidence seems to show that light infections are deleterious and justify thorough control measures. For example, the work of Waite and Neilson[6] showed that the retardation of mental development was definite even in moderately infected children of Queensland. In some very heavily infested countries it may be necessary to limit treatment at first to the most heavily infected occupational groups, but in Australia the public has already health standards sufficiently high to make it unwilling to permit the steady slight damage from hookworm disease to continue, even when the amount of injury is compatible with moderate laboring efficiency.

CONCLUSIONS

1. Hookworm disease is an occupational disease related to mining in two groups of Australian coal mines, but is absent from most of the mines of Australia. A few of the non-infested mines appear to be protected by the salinity of their waters.
2. In Australia hookworm disease cannot, at the present time, be regarded as an occupational disease related to tilling the soil.
3. Hookworm disease in rural Australia is largely limited to areas having over forty inches of annual rainfall, and involves chiefly the school children, affecting the agricultural workers to a lesser extent.

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[6] Waite, J. H., and Neilson, I. L., A Study of the Effects of Hookworm Infection upon the Mental Development of North Queensland School Children, the Medical Journal of Australia, vol. 1, 6th year, pp. 1–8 January 4, 1919.

CONCLUSIONES

1. La uncinariasis es una enfermedad ocupacional con relación a dos de los grupos de las minas carboníferas de Australia, pero no existe en la mayor parte de los campos mineros. Varias de las minas sin infestación parecen estar protegidas por la salinidad de las aguas.

2. La uncinariasis en Australia no puede considerarse como enfermedad de ocupación relacionada con la agricultura.

3. La uncinariasis en la Australia rural está limitada a aquellas areas que tienen una caída de agua anual de mas de cuarenta pulgadas, afectando generalmante a los niños de escuela y en menor grado a los agricultores.

A STUDY ON THE TOXICITY OF CARBON TETRACHLORIDE

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The great interest aroused during these last years by campaigns against intestinal parasites has naturally led to the use of various vermifuges. The experiments with each new drug have aroused high hopes, but the end results have been disappointing, for the free use of each drug has always been followed by the appearance of more or less numerous cases of intoxication.

The work of Darling, Barber, and Hacker, and also the sanitary campaigns undertaken in the interior of the state of São Paulo, Brazil, have demonstrated the advantages of chenopodium oil over thymol and betanaphthol. Until that time these latter drugs enjoyed great popularity, but subsequently they have been used less and less, whereas chenopodium has generally taken their place.

Chenopodium oil has been our best therapeutic weapon against uncinariasis on account of its high anthelmintic power, and despite its toxicity. In the middle of the year 1921, however, the importance of chenopodium threatened to be altogether forgotten and the therapeutic problem of verminosis seemed to have been solved by Dr. Maurice C. Hall's studies on carbon tetrachloride. This author was enthusiastic about the results obtained with this drug, describing the great efficiency and the almost complete innocuity of this new vermifuge.

In view of Hall's splendid results, one of us (Pessôa), in collaboration with Smillie, was led to use the new vermifuge in the treatment of some individuals suffering from uncinariasis. Quite contrary to our expectations, certain difficulties in the free use of the drug arose due to the symptoms manifested by several patients soon after administration of the treatment.

Among the group treated according to Hall's method, and with doses equal to those which he recommends, we saw three cases with severe attacks of pain in the lumbar regions. One of them had marked hematuria, and there were various other symptoms which were felt by other members of the group. This fact led us to suspect that the drug might be toxic even in small doses, for we had followed Hall's recommendations and never gave a dose greater than 3 cc. for an adult.

Two of the persons treated had presented other pathological conditions than uncinariasis, for they had shown signs of alcoholic cirrhosis of the liver. Thus it was apparent that alcoholism might be a factor which would influence the wide adoption of tetrachloride as a vermifuge. Before using this drug further for field studies we took it upon ourselves to verify how far one could apply Hall's conclusions. Experiments, which are detailed in this paper, were made on dogs, to determine the influence of tetrachloride upon the various organs.

The animals experimented upon were taken at random. They were obtained from various places and they seemed all in good physical health if we may judge by the activity they displayed and the amount of fat found in the ''panniculus adiposus.'' To several of them we administered the doses of vermifuge just as soon as they had been admitted to our kennels; others were treated only after remaining in our possession for a variable period of time. These dogs were fed regularly and abundantly and rested for several days. Thus we hoped they might be in better condition to resist the action of toxic substances. In all cases, however, whenever the dose of the drug went beyond a certain limit, which varied in accordance with the weight of the animal, some effects of the carbon tetrachloride became evident. Some animals refused all food after the administration of the treatment.

It was not possible for us to find out the exact age of these animals and we were guided in the administration of the drug principally by the weight and external appearance. This, as we shall see later, instead of militating against our results, brought us certain advantages, for thus we reproduced to a certain degree the circumstances met with in widespread sanitary campaigns.

The drug used in these experiments was obtained from various sources. We used, in experiments 1 to 18, the carbon tetrachloride prepared by Merck. In the subsequent experiments we made use of a sample of the drug prepared by Baker and Co. in the United States. The results were similar in all cases.

Several authors (Andrews, Nunneley, Laffont, Ure, etc.) have noted the toxic properties of carbon tetrachloride. The lesions noted in our experiments led us to compare its action on the animal organism to that of chloroform (Klotz), a drug whose chemical composition is very similar to that of carbon tetrachloride.

Hall himself, who recommends its use, acknowledges its toxicity, for in his paper on "The use of carbon tetrachloride for the removal of hookworms" he refers to an article published in the *Journal of the American Medical Association* (July 31, 1909) which reports the case of a young girl who died of intoxication in consequence of rubbing the scalp with carbon tetrachloride, during a shampoo. It is true, however, that she was the subject of status lymphaticus. In the same paper Hall refers to another article in the *British Medical Journal* (September 25, 1920) which reports another case of intoxication by carbon tetrachloride. This case was observed in a working girl who had handled it for some time in the preparation of golf balls.

OUR EXPERIMENTS

Taking into consideration the facts reported above, we undertook a series of experiments so as to be able to form a personal opinion as to the toxicity of this drug. These experiments are summarized in table 1.

MACROSCOPIC LESIONS

The cases recorded in table 1 and marked with three crosses are those in which the lesions were most severe. In them the

TABLE 1

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	DOSE PER KILOGRAM	DURATION OF TREATMENT	TOTAL DOSE	NUMBER OF TREATMENTS ADMINISTERED	SYMPTOMS	TOTAL DURATION OF EXPERIMENT	DEATH OF ANIMAL	LESIONS In liver	LESIONS In kidneys	OBSERVATION
	kilograms	*cc.*	*days*	*cc.*			*days*				
1	7.2	0.41	6	12.0	4	Tremor, dilated pupil	6	E	+++	+++	Plus ¼ ascaridol
2	6.8	0.58	5	16.0	4	Drowsiness, convulsions	3	S	+++	+++	Plus ¼ ascaridol
3	8.5	1.5	1	12.25	1	Vomiting	3	S	+++	+++	
4	10.4	0.5	3	12.6	3	Jaundice	8	E	+++	++	
5	6.0	0.16	1	1.0	1	Nihil	4	S	0	0	Subcutaneous administration and saline purge
6	5.5	0.36	1	2.0	1	Nihil	4	S	+	0	No purge used
7	7.0	0.44	1	3.0	1	Nihil	4	S	++	0	No purge used
8	9.5	1.0	5	28.5	3	Drowsiness	20	S	+	++	No purge, regeneration of hepatic cells
9	5.9	0.5	1	3.0	1	Nihil	4	S	+	0	Saline purge two hours later
10	3.0	0.5	1	1.5	1	Nihil	4	S	++	0	No purge, young animal
11	5.6	1.0	1	5.6	1	Nihil	4	S	0	0	Emulsion in castor oil
12	7.0	0.28	1	2.0	1	Nihil	4	S	+	++	Subcutaneous administration and saline purge
13	5.5	3.0	1	16.5	1	Intoxication	4	S	+++	+	Emulsion in castor oil
14	3.4	0.2	6	1.3	2	Nihil	20	S	0	++	Liver shows signs of regeneration
15	5.3	0.2	6	2.1	2	Nihil	20	S	0	0	Saline purge in one hour, regeneration
16	5.5	0.2	6	2.2	2	Nihil	20	S	0	0	Emulsion in castor oil. Regeneration
17	4.0	0.05	1	0.2	1	Nihil	10	S	0	+	No purge
18	4.1	0.1	1	4.4	1	Nihil	10	S	+	++	No purge
19	4.2	0.15	1	0.6	1	Nihil	8	S	+	+	

20	4.2	0.3	1	1.26	1	Drowsiness	7	S	+	0	80 grams alcohol one-half hour before CCl_4
21	3.2	0.4	1	1.2	1	Nihil	5	S	+	+	
22	4.15	0.2	1	0.8	1	Nihil	5	S	0	0	
23	6.4	0.05	1	0.32	1	Nihil					
24	5.5	0.05	1	0.27	1	Nihil	6	S	0	++	Saline purge two hours later, old animal
25	7.0	0.05	1	0.35	1	Nihil	6	S	0	++	Emulsion in castor oil
26	6.5	Nihil						S	0	0	Animal killed without treatment

+++, Very marked fatty degeneration (macroscopically visible); ++, marked fatty degeneration; +, slight fatty degeneration; 0, no appreciable fatty degeneration; E, animal died; S, animal was killed (intra-cardiac injection of cyanide of potassium).

macroscopic examination of the liver revealed marked signs of necrosis and fatty degeneration. The examination of the surface of the organ was sufficient to reveal the extensive alterations in the cellular structure of the parenchyma. Instead of its normal aspect the color of this liver was bright yellow and lusterless, and the whole organ showed a trabecular tracery formed by small clear yellow lines enclosing dark, depressed, punctiform zones. The surface of the cut section showed a similar pattern. In certain cases the liver was darker and the lesions were less clear; in others the contrast between the areas of fatty degeneration and the red areas of necrosis was not as clear, although the punctiform and multiple retractions of the capsule of Glisson in the center of the hepatic acini resulting from an acute central degeneration of the lobules, were visible on the external surface of the organ.

In the kidneys the external appearance was not markedly abnormal. The transverse section made in the direction of the longest diameter always showed the same unusual characteristics. The outer surface of the organ extending from one pole to the other showed a brownish yellow zone within the area of the convoluted tubules. The more external portion of the cortex showed a sort of greyish band of linear threads radially arranged and placed very near each other. This zone of cloudy swelling and fatty degeneration was much more marked in some of the cases and, although not always present, it was most marked in the cases in which, besides this lesion, there was a congestion of the organ.

One of the dogs which was found dead after treatment showed, besides the lesions already described in the liver and kidneys, evident signs of icterus with numerous foci of biliary pigment scattered throughout the whole organ. The urine of this animal contained large amounts of bile.

MICROSCOPIC LESIONS

The microscopic lesions also varied in intensity. In the more severe cases there was always found in the liver a zone, located at the periphery of the acini, where the hepatic cells showed great infiltration by droplets of fat. These cells still retained their normal relation within the columns of the acini. The central portion of the lobules however, showed a marked but varying necrosis in which were found cell debris, nuclear remains, extravasated erythrocytes and a few drops of fat, smaller than found at the periphery.

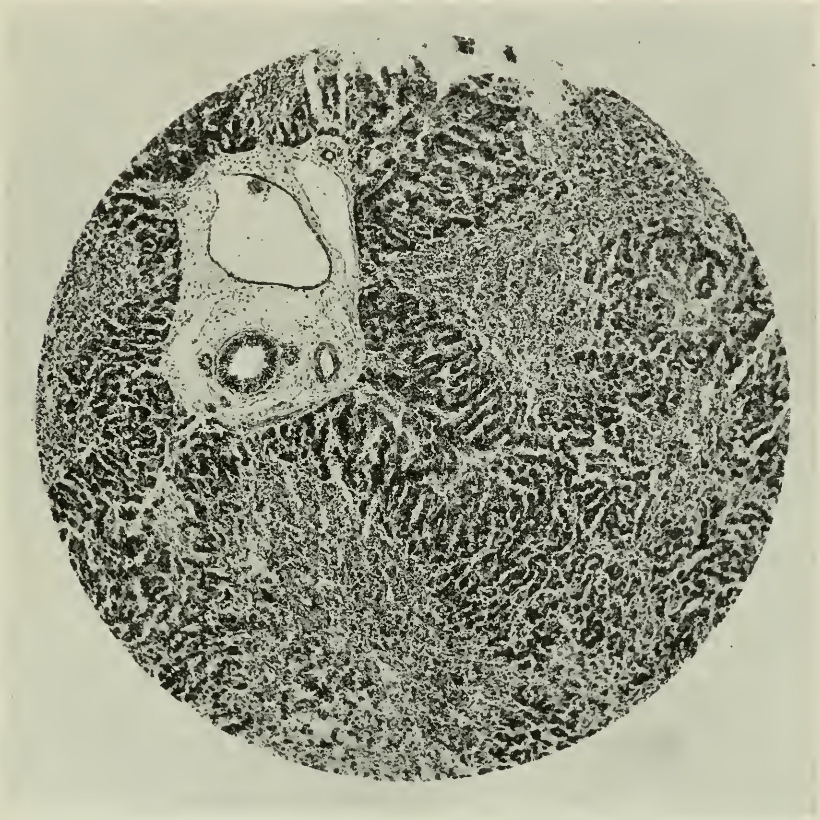

Fig. 1. Experiment 3, Table 1

In the animals in which the central necrotic process was not so advanced the hepatic trabeculae of the central zone lost their normal characteristics and showed a sort of collapse. The evidences of central congestion were constant in specimens of this sort. At other times where the intoxication was slight the microscopical changes of the liver were limited to the presence of fat droplets of various sizes, localized in the vicinity of the portal spaces. All of the lesions of different grades of intensity bore a close resemblance to those which may be produced in dogs by chloroform.

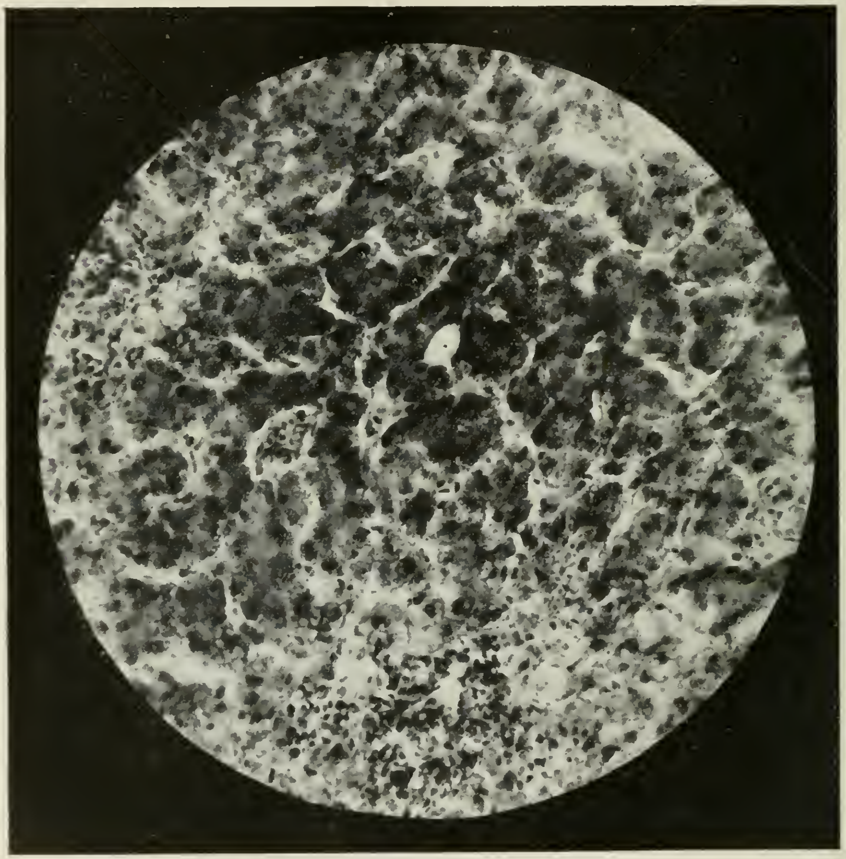

Fig. 2. Experiment 13, Table 1

In those experiments in which the interval of time between the administration of the vermifuge and the day on which the animal had been killed or had died, was longer, the fat droplets in the liver cells had almost completely disappeared. When stained with Sudan III, the parenchyma still revealed the presence of fat droplets, but the number of them was quite small. On using this stain the tissue took on a clear and transparent appearance.

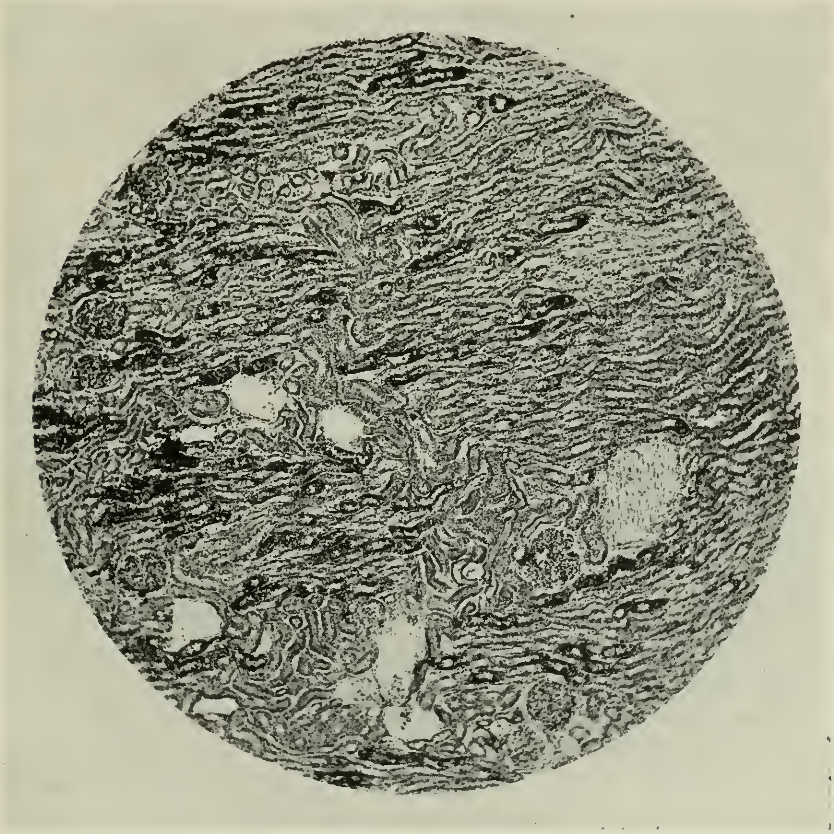

Fig. 3. Experiment 18, Table 1

On this background was seen, in the cases where the treatment had been less recent, a new stroma reproducing the normal structure of the organ.

In the kidneys the microscopic alterations consisted in the appearance of fat drops in the protoplasm of the epithelial cells of the convoluted tubules and of the loops of Henle. These fat drops were found in the inner and freer portions of the cytoplasm so that they frequently hid the nucleus by compressing it against the border of the supporting stroma. In a few cases we noted a small amount of congestion of the glomeruli. When the administration of the drug had been a considerable time antecedent or in moderate doses, the amount of fat was less but in greater amount than was seen in the control animals, as shall be reported further on.

The dogs which had received the largest doses of the drug showed the most marked lesions.

CONTROL EXPERIMENT

As a control to these studies we killed several animals without having administered the vermifuge to them.

The macroscopic examination of the liver and kidneys did not reveal any fact worthy of note.

Microscopically the liver, after staining with Sudan III, revealed some fat droplets not in form of fat globules but rather as a very fine spray of minute droplets, in the neighborhood of the biliary canaliculi, and not in the vicinity of the hepatic blood spaces. We assumed them to be normal. There was no evidence of central degeneration in the lobules. In the renal parenchyma we found a few small fat globules besides occasional signs of an interstitial nephritis and the presence of small adenomas of suprarenal origin. The amount of fat found in no way compared with the amounts noted in the cases to which the vermifuge had been administered. In the case where no drug had been administered only two or three fat globules were found in each microscopic field, whereas in the other cases a large portion of the cortex of the organ was altered.

Moreover none of the untreated cases showed degeneration or necrosis either in liver or kidney.

DISCUSSION AND INTERPRETATION

Among the various questions raised by our findings there are several which deserve our attention on account of the greater or lesser weight they seem to have on the question of the action and toxicity of the drug studied.

It was not possible for us to obtain dogs of the same age, breed, or weight, so that in each experiment we were obliged to observe the effect of these factors in relation to the treatment and to the effect of the drug as regards its value as a vermifuge and in respect to its toxic influence. This, besides rendering our interpretation more difficult, made it impossible for us to consider each case by itself. For this reason we grouped the results in the following analysis. We do not feel that this fact should minimize the value of our conclusions, for by taking the cases just as they came, we reproduced up to a certain degree the conditions met in sanitary campaigns, for the hygienist who plans the treatment of whole populations can only make use of general data such as age, sex, physical appearance, etc., for the adoption of the adequate dosage, or as a criterion in the judging of the advantages or disadvantages of a certain treatment.

Thus, by using doses which varied between 0.05 to 3 cc. per kilo of animal weight in one, two, three or more treatments, with or without purge, we observed that the same amounts of the drug could produce different effects, depending on the concomitant action of other factors. The most striking of these factors is that of the age of the animal. The animals with greater weight and development and those which seemed to us to be the older ones, showed a marked susceptibility to the drug. The toxic effects of the drug on the liver could be rendered less severe only by the use of a saline or oil purge. In some cases however, even with the use of a purge after doses of 0.05 to 0.1 cc. per kilogram weight (experiments 17 and 18) we would note lesions ten days after the treatment.

The favorable effects of a purge, such as have already been mentioned in the case of adult animals, were also evident in the case of younger ones. Thus young dogs to which 0.4 to 0.5 cc. per kilogram weight had been administered in one or two treatments with subsequent purge showed no evidences of pathological changes in the kidneys (experiments 15 and 16) and only one case (experiment 5) showed slight lesions in the liver.

In accordance with the facts just reported we believe that a purge does not completely protect older animals against the toxic effects of even small doses of carbon tetrachloride. Two of our experiments are rather suggestive as to this point (experiments 24 and 25). An interesting fact is the relation between the varying intensity of the lesions in the kidneys and the longer or shorter period elapsing after the treatment. Broadly speaking, it appeared that the kidney lesions were slower in making their appearance, so that the most marked change occurred when some days were allowed to elapse between the time of administration of the drug and the death of the animal. Although no renal lesions were observed in two of our cases (nos. 15 and 16) and this seems to have been due to the use of the purge which we administered in these treatments.

This seems to be even more evident when we consider that in another case (no. 4) the lack of purge was sufficient to permit the appearance of the kidney lesions after the administration of an equal dose of the drug. The renal lesions were most marked in another animal twenty days after the administration of relatively large doses of the vermifuge without a purge, although the lesions in the liver showed signs of regeneration (experiment 8).

In some of the other cases the renal lesions were of moderate intensity, or almost absent when the histological examination took place five, six, seven, eight days after the treatment.

The mode of administration of the vermifuge is a question of some importance. In one experiment (no. 5) we injected subcutaneously 1 cc. of carbon tetrachloride and then administered a saline purge. No lesions whatever were noted either in the liver or in the kidneys. Knowing that the therapeutic effect of this agent depends upon its poisonous action on the intestinal parasites on which it seems to act by direct contact, the hypodermic method of administration would not be effective, since the drug is not entirely eliminated by the intestines. Since the worms are localized in the intestinal tract it becomes evident that the action of carbon tetrachloride will be all the more efficient the more directly it acts upon the parasites.

It is evident that the action of the drug on the different organs results only after its absorption from the intestinal mucosa. The best way to prevent its toxic action would be to render this absorption impossible.

In an endeavor to find out the influence of alcohol in regard to the greater or lesser severity of the lesions produced, we administered 80 grams of ethyl alcohol per os to a dog (experiment 20), half an hour before the administration of a therapeutic dose of the vermifuge. We expected to find more marked lesions whereas to our surprise we found after seven days only slight signs of degeneration in the liver and only slight changes in the kidneys. To confirm this fact we administered a somewhat smaller dose of carbon tetrachloride (experiment 24) without a purge, and found after five days signs of regeneration in the liver together with slight lesions in the kidneys. This is a proof of previously existing lesions. The fact that the regeneration had not been so complete in the previous experiment (no. 20) when the histological examination took place seven days after the administration of the drug, whereas in experiment 22 the examination was made five days afterwards, may be explained by the fact that alcohol exerts a selective action on the hepatic cells, altering them or preventing the disposal of the fats resulting from the pathological process. Further work is necessary on the relation of the use of alcohol to the degree of toxicity of the drug.

A point worthy of notice is the way in which the regeneration of the parenchyma takes place. The process of necrosis nearly always occurs in the central portion of the lobules. This leaves the peripheral cellules intact and they provide for the needed new growth. However, other phenomena occur before this takes place. After the period of congestion is over, the blood vessels from which there has been an escape of blood, leave many blood corpuscles scattered in their neighborhood. The presence of hemosiderin can be demonstrated.

The short period of time occupied by the treatment and our desire to study the acute lesions prevented us from investigating the possibility of the occurrence of chronic progressive lesions after the use of this drug, such as can be produced by ammoniacal copper acetate and other substances. However, taking into account the fact that the hepatic epithelium is one of the delicate tissues of the body, and considering the multiplicity of its functions, it is evident that it suffers a much greater destruction than the stroma which supports it. For this reason, an experimental cirrhosis may result from repeated experiments even though small doses are used, by the same mechanism which explains the cirrhosis produced by chloroform or the salts of copper or lead.

The fat which appears with the degenerative lesions of the liver disappears gradually, and in those cases showing regeneration it is seen as a fine spray.

The process by means of which the fat in the kidneys disappears is a somewhat different one. As we have already said in describing the microscopic lesions, the fat does not occupy the basal portion of the cellular protoplasm, but rather the part nearest to the lumen of the tubules where, at times, on account of their numbers, at others on account of their size, the fat drops compress the nucleus against the supporting stroma. It is very common to find a great number of fat drops of all sizes. At times these drops are also found in the inter-cellular spaces. More frequently, however, they occupy the interior of the tubules, where they seem to have been secreted. It seems therefore, that in the kidneys the elimination of the fat, in contrast to what is observed in the liver, is not brought about by a process of absorption, but through a process of elimination. This seems the more probable when we remember that in a case of experimental fatty degeneration, A. Fourleton noted the presence of casts of solid fat, filling and molding itself within the cavity of the renal pelvis and tubules.

The conclusions of Hall, Shillinger, Lake, and Allen are somewhat opposed to the ones we drew from our experiments. The first of these authors, after repeated experiences with this drug, concluded that its use caused no inconvenience when administered in doses of 0.3 cc. per kilogram of animal weight (dog). Hall recommended afterward a dose of 0.05 cc. per kilo of body weight for man. After undertaking new experiments in collaboration with Shillinger and Lake, he concluded that doses 10, 20, 30, or even 40 times greater than those advised in his first communication are non-toxic for monkeys (the experiments consisted of 12 treatments with doses of 1 to 4 cc. of carbon tetrachloride, administered at intervals of one to two days, during a period of one month). He states that these experiments indicate the apparent safety of the drug for human beings.

It is not possible to accept Hall's conclusions, without some reservation, on account of the results which these same authors report and which we had occasion to confirm.

Despite these optimistic conclusions as to the non-toxicity of the drug, one of these authors (Lake) reports in his article that of the four monkeys treated, two vomited after the treatment and that another monkey expelled a much larger amount of feces than normally during the second and third week of the treatment. Lake tells us that the liver, the kidneys and the intestines were always examined in the later experiments, although Hall did not make a microscopical examination of the internal organs in the first ones. Lake says that despite all the care exercised in these examinations, no lesions were ever found in these organs, such as could be attributed to the effects of carbon tetrachloride.

The question arises as to the possible hypersensitiveness of dogs to this drug, in comparison to the resistance claimed for anthropoids. Such a conclusion can hardly be drawn from the work of different authors when we remember that Lake reported in his article (*Public Health Reports*, 1922), that Hall and Shillinger administered to some dogs amounts as large as 300 cc. of carbon tetrachloride in a single dose without producing any symptoms of intoxication. We do not know why it is that after using only about 1 cc. of carbon tetrachloride, which we believe had been prepared by the same makers, we twice observed lesions in the liver and once in the kidneys (experiments 19 and 20).

As can be seen, there exists some divergence in the results observed, but our own results led us to believe that we should use care in the administration of carbon tetrachloride in extensive campaigns against uncinariasis.

The exact mechanism by which the central degeneration and necrosis of the liver lobules is brought about is yet a controverted point, and is explained by different authors in different ways. Comparing the facts observed in this intoxication with those noted in intoxications caused by phosphorus and chloroform we are led to believe that this degeneration results from an autolytic process in the hepatic cells. According to Flexner and other authors, the oxidizing enzymes (aldehydase) acting on the hepatic parenchyma cause the appearance in the urine and blood of large amounts of substances resulting from the splitting up of the albuminoids. These substances in turn lessen the coagulability of the blood and cause hemorrhages. This theory seems to be confirmed by the increase of amino-acids in the blood and urine in these cases. Similar facts have repeatedly been demonstrated in cases of eclampsia and chloroform intoxication. The authors who made these observations in cases of intoxications resulting from the administration of anesthetics, noted that the functional activity of the hepatic cells was suspended and their vitality even destroyed, whereas the enzymes continued to display the same activity as previously and lost none of their properties. A similar result may take place with bacterial toxins. The result of this activity leads to cellular disintegration in consequence of the action of the enzymes upon the protoplasm of the cells.

Some authors, and among these Evarts Graham, consider the lesions of the cells in the parenchyma to be caused by the action of the hydrochloric acid formed in the liver.

The kidney lesions, which are always less severe than those of the liver, probably result from the direct action of the drug during its excretion through the tubules.

VALUE AS A VERMIFUGE

The results of our experiments as to the value of carbon tetrachloride as a vermifuge, agreed in every detail with those of Hall. The value of carbon tetrachloride as a vermifuge can be deduced from the facts reported in table 2. The number of worms expelled by a single treatment with carbon tetrachloride is found in columns 6 and 7, whereas the two last columns give the number of worms not expelled by this treatment but encountered at the post-mortem examination.

TABLE 2

First treatment

NUMBER OF DOG	WEIGHT	DRUG USED	DOSE PER KILO	PURGE	WORMS EXPELLED		WORMS NOT EXPELLED	
					Ancylostomes	Others	Ancylostomes	Others
	kgm.		*cc.*					
1	7.200	CCL_4, ascaridol	0.41	Yes	11	0	0	0
2	5.800	CCL_4, ascaridol	0.58	Yes	1	0	0	0
3	8.500	CCL_4	1.50	Yes	71	0	0	0
4	10.400	CCL_4	0.50	Yes	9	1 ascaris	0	0
5	6.000	CCL_4	1.60	No	0	0	0	0
6	5.500	CCL_4 (inj.)	0.36	No	0	0	0	0
7	7.000	CCL_4	0.44	No	3	0	0	0
8	9.500	CCL_4	1.00	No				
9	5.900	CCL_4	0.50	Yes	3	0	0	0
10	3.000	CCL_4	0.50	No	0	1 ascaris	0	0
11	5.600	CCL_4	1.00	Emulsion in oil	4	0	0	0
12	7.000	CCL_4 (inj.)	0.28	Yes	0	0	40	0
13	5.500	CCL_4	3.00	Emulsion in oil	7	0	0	0
14	3.400	CCL_4	0.20	No	0	0	0	0
15	5.300	CCL_4	0.20	Yes	1	0	0	0
16	5.500	CCL_4	0.20	Emulsion in oil	4	0	0	0
17	4.000	CCL_4	0.05	No	0	0	15	0
18	4.100	CCL_4	0.10	No	0	0	3	0
19	4.200	CCL_4, ascaridol	0.25	No	0	0	0	0
20	4.200	CCL_4, alcohol	0.30	No				
21	3.200	CCL_4	0.40	No				
22	4.150	CCL_4	0.20	No	0	0	0	0
23	6.400	CCL_4	0.05	Yes	0	0	2	8 ascaris
24	5.500	CCL_4	0.05	Yes	0	0	0	0
25	7.000	CCL_4	0.05	Emulsion in oil	0	0	14	1 dipylidium

Carbon tetrachloride is efficient in doses of 0.3 cc. per kilogram weight of animal; it has little vermicidal action in smaller doses. It does not close its action when emulsified in castor oil.

MODE OF ACTION OF THE VERMIFUGE

Carbon tetrachloride acts directly on the worms during its passage through the intestines. A therapeutic dose of carbon tetrachloride given subcutaneously to dogs and followed in a half hour by a saline purge did not cause the expulsion of any worms, although we found 40 ancylostomes in the intestines at the post-mortem examination of the dog (no. 12). This was to be expected, since carbon tetrachloride appears to be eliminated through the kidneys and the lungs. Thus it could exert no action on the intestinal mucosa, and consequently none also on the worms attached to it.

CONCLUSIONS

It seems reasonable to draw the following conclusions from the facts reported above:

1. Carbon tetrachloride is a substance which exerts a toxic action on the animal organism (dogs) when administered by mouth.
2. The most marked lesions are observed both by macroscopic and by microscopic examination, in the liver and in the kidneys. The severity of these lesions seems to vary in inverse ratio to the age of the animal.
3. The severity of the liver necrosis is in direct proportion to the dose administered, although we occasionally observed a case of hypersusceptibility to carbon tetrachloride.
4. The liver necrosis appears to be brought about by the same mechanism as that from chloroform intoxication.
5. The renal lesions are less severe than those in the liver and develop more slowly.
6. Both liver and kidneys show active power of regeneration to replace the destroyed parenchyma.
7. Carbon tetrachloride is a powerful vermifuge. When administered in doses of 0.3 cc. per kilogram of body weight it removed all ancylostomes in dogs.
8. The administration of this vermifuge should be undertaken with caution in the treatment of human beings and the doses recommended by Hall may be too large.

CONCLUSIONES

De los hechos y observaciones ampliamente consideradas en el artículo que precede, desprenden los autores las siguientes conclusiones:

1. El tetracloruro de carbono es una substancia que ejerce una acción tóxica en el organismo animal (perros) cuando se administra oralmente.
2. Las lesiones se observan macroscópica y microscópicamente, en el hígado y en los riñones. La severidad de las lesiones varía en relación inversa con la edad del animal.
3. La severidad de necrosis hepática existe en relación directa con la dosis administrada, aunque ocasionalmente se observan casos de hipersusceptibilidad al tetracloruro de carbono.
4. La necrosis hepática se debe al mismo mecanismo que la intoxicación clorofórmica.
5. Las lesiones renales son menos severas que las del hígado y se desarrollan más lentamente.
6. Ambos hígado y riñones demuestran gran poder regenerativo del parénquima.
7. El tetracloruro de carbono es un vermífugo poderoso. En dosis de 0.3 c.c., por kilogramo de peso extirpa todos los anquilostomas en el perro.
8. La administración de este vermífugo debe emprenderse con cautela en el humano y en dosis mas pequeñas que las que recomienda Hall.

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THE EFFECT OF CARBON TETRACHLORIDE ON INTESTINAL PROTOZOA

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Since the use of carbon tetrachloride as an anthelmintic is becoming so general the question has naturally arisen as to what its effect is on the intestinal protozoan parasites of man.

THE EFFECT OF CARBON TETRACHLORIDE ON INTESTINAL PROTOZOA IN CULTURE

In the fall of 1921 one of the authors[1] had in the laboratory cultures of *Trichomonas hominis*, *Embadomonas intestinalis* and *Spirochaeta eurygyrata*. They were all living on a culture medium known as sodium chloride serum water (Hogue (1)). This is a combination of 0.85 per cent sterile solution of sodium chloride and either sheep or pig serum water made up with sterile distilled water in the proportion of 1 part of serum to 3 or 4 parts of distilled water.

Several large loopfuls of these cultures were put on slides and a small drop of carbon tetrachloride from a capillary pipette was added to each slide. The organisms were almost immediately killed. In the case of *Trichomonas hominis* the animals rounded up and activity ceased. The flagella moved for a few seconds and then separated from the body and soon went to pieces. The body wall broke down and the animal completely disintegrated. *Embadomonas intestinalis* behaved in practically the same way as *Trichomonas hominis*.

In the case of *Spirochaeta eurygyrata* the organisms became inactive, but did not disintegrate. This is characteristic of these spirochetes. The dead forms are found intact in the cultures long after all activity has ceased (Hogue (2)).

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[1] The work on animal experimentation was done by Hogue in the Department of Pathology and Bacteriology of Johns Hopkins University.

EFFECT OF CARBON TETRACHLORIDE ON INTESTINAL PROTOZOA IN KITTENS

The next step was to inoculate animals with intestinal protozoa or to find those carrying protozoan infections. Several unsuccessful attempts were made to inoculate cats, kittens and rabbits with *Trichomonas hominis* (Hogue (1)) and to inoculate kittens with *Spirochaeta eurygyrata* (Hogue (2)). Fortunately three kittens were found, each of which was infected with Giardia, spirochetes and Bellascaris. These kittens varied in weight from 640 to 825 grams. The first kitten was given 0.2 cc. of carbon tetrachloride and still continued to pass Giardia cysts and spirochetes. It died five days later. The autopsy showed an obstruction in the large intestine.

	MAY 1	MAY 4	MAY 8	MAY 11	MAY 19	MAY 27	JUNE 6
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Kitten 1........................	0.2						
Kitten 2........................		0.5	1.0		1	1	1
Kitten 3........................	0.2	0.5	0.5	1	1	1	1

The second kitten received doses of 0.5, 1, 1, 1, 1 cc., in all 4.5 cc. of carbon tetrachloride. Daily fecal examinations were made. On May 5, Giardia, spirochetes and Bellascaris were found in the stools. From May 5 to May 18 the stools were free from active protozoa, though a few inactive spirochetes were passed on May 12. On May 18, Giardia cysts were passed and on May 19 both Giardia cysts and active spirochetes. On May 20, following the third dose of carbon tetrachloride, Giardia cysts were passed. Spirochetes were passed at irregular intervals. Giardia cysts again appeared in the stools on June 6, and were found there daily until June 9 when the experiment was stopped.

The third kitten continued to pass Giardia cysts and spirochetes from May 1 to May 10 in spite of the treatment with carbon tetrachloride which in this case had been in smaller amounts, i.e., 0.2 0.5, 0.5 cc. On May 11 it received 1 cc. and did not pass Giardia cysts until May 19. It was then given 1 cc. of carbon tetrachloride. It continued to pass Giardia cysts and spirochetes at irregular intervals until the experiment was closed on June 9.

It is evident from these experiments that 1 cc. of carbon tetrachloride is not a therapeutic dose for removing Giardia cysts and spirochetes from kittens, though Hall (3) has shown that 0.3 cc. per kilo of weight is sufficient to remove hookworms from dogs.

EFFECT OF CARBON TETRACHLORIDE ON INTESTINAL PROTOZOA IN MAN

In October, 1922, we started treating intestinal protozoan infections in man with carbon tetrachloride. As a rule the dosage used has been 3 cc. of carbon tetrachloride in a small amount of water. It has been given on an empty stomach as recommended by Lambert (4) and Smillie and Pessoa (5). As a rule it was followed three hours later with a dose of epsom salts. In a few cases the salts were omitted or given later but this did not seem to affect the patients. The following is a report of some of the cases treated.

Case 1. Mr. A., a married white man, forty-four years old, was admitted to the hospital November 12, 1922. He was in an emaciated condition. The diagnosis was acute appendicitis, and he was operated on for this. His stools were unformed. A microscopic examination showed large numbers of both the cysts and active forms of *Chilomastix mesnili.* On November 23 he was given 3 cc. of carbon tetrachloride which was followed three hours later with a dose of epsom salts. On November 25 he was still passing loose stools containing cysts and active forms of *Chilomastix mesnili.* He was then given another dose of 3 cc. carbon tetrachloride. After that the stools were formed though he continued to pass the cysts, but not the active forms of *Chilomastix mesnili.* He left the hospital December 3, 1922.

Case 2. An unmarried white woman, age twenty, was found infected with *Trichomonas hominis.* She was given 3 cc. carbon tetrachloride in the morning and at three in the afternoon was given a dose of epsom salts. Her stools have been examined at irregular intervals since then and have always been found to contain *Trichomonas hominis.*

Case 3. An unmarried white woman, age twenty, was found carrying infections of *Giardia intestinalis, Trichomonas hominis,* and *Iodamoeba williamsi.* December 15 she was given 3 cc. of carbon tetrachloride and the following morning a dose of epsom salts. After that the stools were examined microscopically and culturally. The cysts of *Giardia intestinalis* and *Iodamoebe williamsi* were always found in the stools and *Trichomonas hominis* appeared in the culture tubes of sodium chloride serum water which had been inoculated with the fecal material.

Case 4. A white girl, nine years old, was brought to the hospital May 11, 1922 from a children's home. She was much under size and quite anemic. In October a stool examination was made and she was found to be infected with *Giardia intestinalis, Endolimax nana, Trichomonas hominis* and hookworm. On October 27 she was given 0.5 cc. of carbon tetrachloride but continued to pass *Trichomonas hominis* and cysts *of Endolimax nana* and *Giardia intestinalis.* On October 30, and again on November 2 and November 7 she received 0.5 cc. of carbon tetrachloride but continued to pass Trichomonas and cysts of Giardia and *Endolimax nana.* After the last dose the stools were crowded with cysts of *Endolimax nana* and hookworm eggs. On November 10 the dose was increased to 1 cc. On November 17, she was given 1.5 cc. After both of these doses the cysts of Giardia and *Endolimax nana* disappeared though Trichomonas was still present. On November 25, cysts of Giardia and *Endolimax nana* were again present and Trichomonas appeared in the culture tubes inoculated with fecal material. On November 28, and again on December 13, 3 cc. of carbon tetrachloride were given and followed by doses of epsom salts. The patient continues to pass cysts of *Giardia intestinalis* and *Endolimax nana* at irregular intervals though *Trichomonas hominis* always appears in the culture tubes of sodium chloride serum water inoculated with fecal material. It is interesting to note that the hookworm infection is most persistent. The eggs are still found in the stools in spite of the many doses of carbon tetrachloride; in all, a total of 10.5 cc.

DISCUSSION

From the foregoing experiments it seems evident that carbon tetrachloride has no permanent effect on intestinal protozoan infections. Infections of *Trichomonas hominis* and *Chilomastix mesnili* persist in spite of the full dosage, i.e., 3 cc. Infections of *Giardia intestinalis* appear at times to be affected Cysts will disappear from the stools for a few days after treatment. However, we must remember that this is characteristic of *Giardia infections.* Various authors have already shown that the cysts of Giardia appear in the stools at irregular intervals, often several days apart so that their disappearance from the stools of these patients under treatment cannot be taken as due to the effect of carbon tetrachloride. The same appears to be true for *Endolimax nana* though less is known about this infection.

CONCLUSIONS

1. Carbon tetrachloride in doses of 1 cc. is not specific for the treatment of infections of Giardia and spirochetes in kittens.
2. Carbon tetrachloride in 3 cc. doses is not a specific for the treatment of human infections of *Giardia intestinalis, Trichomonas hominis, Chilomastix mesnili, Endolimax nana* and *Iodamoeba williamsi.*

DISCUSIÓN

Los experimentos que anteceden demuestran que el tetracloruro de carbono no tiene efecto permanente sobre las infecciones protozoicas del intestino. Las infecciones por el *Trichomonas hominis* y el *Chilomastix mesnili* persisten a pesar de la dósis máxima de 3 c.c. Las de *Giardia intestinalis* parecen ser afectadas a veces. Las formas quísticas de los protozoos desaparecen de la excreta a los pocos días despues del tratamiento. Sinembargo esto es caracteristico de las infecciones de Giardia. Varios autores han demostrado que las formas quísticas de Giardia aparecen en la excreta a intervalos irregulares, a veces con mediación de varios días, de manera que su desaparición de la excreta de esta clase de pacientes no puede ser debida al efecto del tetracloruro de carbono. Igualmente ocurre con la *Endolmax nana* aunque poco sabemos acerca de esta infección.

Las conclusiones presentadas son:

1. El tetracloruro de carbono en dosis de 1 c.c. no es específico en el tratamiento de infecciones causadas por Giardias y espiroquetes en los gatos.

2. El tetracloruro de carbono en dosis de 3 c.c. no es específico en el tratamiento de infecciones humanas por *Giardia intestinalis, Trichomonas hominis, Chilomastix nana, Endolimax nana* y *Iodmoeba williamsi.*

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STUDIES ON THE ETIOLOGY OF BLACKWATER FEVER

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Despite numerous investigations on blackwater fever the etiology of this disease is still unknown. One school of investigators believes that quinine and malaria are the factors responsible for the disease. The other school contends that there are two distinct disease conditions, one associated with malaria which they designate as quinine hemoglobinuria; the other specific blackwater fever. The first mentioned condition may be produced in cases of chronic malaria by even minute doses of quinine, while the second has a specific etiology, the nature of which is still unknown.

The mechanism of the production of hemoglobinuria during malaria has been studied extensively by Barratt and Yorke (1909). They found that quinine, hydrochloric acid, and sodium hydroxide will induce the necessary hemolysis, but that during life quinine never reaches a concentration sufficient to cause hemolysis. The anuria they attribute to a mechanical blocking of the kidney tubules by the deposited hemoglobin.

In the course of the last year we have had occasion to study 4 cases of clinical blackwater fever. Certain experiments were made with the hope of obtaining facts with regard to the cause for the appearance of blood in the urine. The purpose of this paper is to report briefly such observations as appear to be of interest.

RELATIONS TO MALARIA AND QUININE

All the cases gave a history of malaria. Malaria parasites were found in the blood of each either before or after the attack of hemoglobinuria. During it no parasites were found. Three of the cases had tropical malaria (2 with gametes) and in the fourth the benign tertian parasite was found. Two of them possess special interest because the attacks occurred in the hospital after the injection of relatively small doses of quinine and the development of the condition could be followed from the beginning.

A brief description of these two is given below:

S. H., girl, fourteen years. Admitted January 21, 1921. Only ten months in the country. Had repeated attacks of malaria during six months prior to admission. Quinine treatment irregular. Came to hospital because of fear of blackwater fever. Blood examination showed tropical gametes. On January 22, 1921, quinine injections were started, 0.05 gram, 8.45 a.m.; 0.5 gram, 11 a.m.; 0.5 gram, 6 p.m During the night the patient had an attack of blackwater fever: temperature 38.3°C., pulse 120; body and sclerae jaundiced; urine red. Examination of urine: hemoglobin+; albumin±; bile —; red cells —; epithelial cells and casts +. The next day the urine was clear and the temperature returned to normal. Quinine was later given without the development of hemoglobinuria. The patient is now well.

R. L., girl, sixteen years. Admitted March 15, 1921. Repeated attacks of malaria during past year. Quinine treatment irregular. Had two attacks of hemoglobinuria, the last two weeks before entering the hospital. One week after admission to the hospital she received a dose of 0.4 gram of quinine on two successive days and 0.5 gram on the third day. A few hours after the last injection the patient developed hemoglobinuria. Temperature 38.6°C.; pulse 120; body and sclerae jaundiced; urine deep red. Examination of the urine revealed the presence of hemoglobin, albumin, white and epithelial cells and casts. Red cells were rare. No malarial parasites could be found in the blood at this time, but four weeks later, while still in the hospital, the patient had an attack of malaria and parasites of the tertian variety were found.

The history and findings of these two cases are typical of the rest. The attack was characterized by a rise in temperature with a corresponding increase in pulse rate and dark red urine containing hemoglobin, albumin, and casts, but practically devoid of red cells. As the urine cleared, the temperature fell and the condition of the patient improved.

SECRETION OF QUININE

In one case a series of quantitative tests was made of the secretion into the urine of quinine and albumin during the course of the attack. Each specimen passed was examined for the presence of these substances. The results are shown in the table. Unfortunately one specimen was lost and consequently the total quinine excreted could not be determined.

It is of interest to note that the concentration of quinine and albumin in the urine roughly paralleled each other, though albumin and blood continued to be eliminated after tests for quinine were negative. Bile was never present.

*Analysis of urine of blackwater fever patient**

SAMPLE	AMOUNT	SPECIFIC GRAVITY	ALBUMIN	QUININE PER CUBIC CENTIMETER	TOTAL	HEMOGLOBIN	BILE
	cc.		*per cent*	*mgm.*	*mgm.*		
1	80	1.0107	0.18	0.1	8.0	+	–
2	(?)	(?)		(?)	(?)	+	–
3	150	1.010	0.36	0.5	75.0	+	–
4	420	1.009	0.24	0.03	12.6	+	–
5	333	1.008	0.12	0.02	6.6	+	–
6	400	1.008	0.12	0.00	0.0	+	–
					102.0		

* The total quinine injected just before the attack was 500 mgm.

FRAGILITY OF RED CELLS IN BLACKWATER FEVER

A number of observers have attributed the destruction of red cells in blackwater fever to alterations in surface tension. Tests were therefore made to determine the fragility of the cells to hypotonic solutions shortly after the attack of blackwater fever. One drop of defibrinated blood was added to each of a series of tubes containing 1 cc. of salt solution of graded hypotonicity. No difference was observed between the behavior of a normal blood and of that from the blackwater fever patients. In both instances marked hemolysis (about 60 to 70 per cent) occurred after fifteen minutes in a salt concentration of 0.27 per cent, and practically none in one of 0.36 per cent.

EFFECT OF QUININE ON BLOOD CELLS FROM MALARIA AND BLACKWATER FEVER PATIENTS AND FROM NORMAL INDIVIDUALS

These experiments were a repetition of those made by Barratt and Yorke and confirmed their findings. Quinine had the same effect on normal and malarial blood. It required a concentration of 0.2 per cent of quinine hydrochloride to cause laking of each. In one series of tests only was the blood of a blackwater fever patient laked by a somewhat lower concentration (0.15 per cent) of quinine. But to attain even this concentration in the body, it would be necessary to inject into the circulation approximately 7 grams of quinine and maintain that concentration for some time.

EFFECT OF BILE ON NORMAL, MALARIAL, AND BLACKWATER FEVER BLOOD CELLS

Subacute jaundice was regularly present in the individuals prior to the attack of hemoglobinuria. The physicians of Palestine consider the jaundice as an indication of danger and refuse to give quinine to such patients. This fact, together with the known hemolytic power of bile, suggested that this substance might play some causative rôle in hemoglobinuria. Tests were made, therefore, on the effect of ox bile, on blood cells of normal individuals as well as of those suffering with malaria or blackwater fever. The results showed that bile in a dilution of 1:25 caused prompt laking of cells of healthy or diseased individuals, while a dilution of 1:50 usually did not lake the blood cells. In either dilution the effect was practically the same on various types of blood.

EFFECT OF MIXTURE OF QUININE AND BILE

Experiments were now made to ascertain the associative action of bile and quinine in various proportions. Interesting results were obtained. Mixtures containing a 1:50 or 1:75 dilution of bile and 0.02 to 0.03 per cent of quinine invariably brought about laking, while the same concentrations of these substances acting separately had no effect. In other words concentrations of bile and quinine, separately incapable of producing laking, caused marked hemolysis when acting in combination. These experiments were repeatedly made with human red cells both normal and malarial as well as with rabbit cells, always with the same results. The following are type experiments indicating the method of procedure, concentrations, and results.

Experiment 1. Ox bile was diluted with saline in the proportion of 1:9 and varying quantities added to a series of tubes containing the same amount of quinine. The volume in each case was made up to 1 cc., and 0.1 cc. of defibrinated blood added to each tube. The tubes were placed in the incubator for one hour and then observed at room temperature.

Quinine mgm......	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.0
Bile (1:10), cc.....	0.1	0.2	0.3	0.4	0.5	0.6	0.0	0.3
Results..........	No hemolysis	Hemolysis in ½ hour	Immediate hemolysis				No hemolysis	No hemolysis

Experiment 2. Same procedure as experiment 1, except bile concentration constant and quinine varied.

Quinine mgm.......	0.4	0.2	0.1	0.05	0
Bile (1:25) cc.......	1.0	1.0	1.0	1.0	1
Results.............	Hemolysis in five minutes		Hemolysis in in ten minutes	Complete hemolysis one hour	Slight hemolysis one hour

Experiment 3. Effect of bile and quinine on blood of malaria patient. Blood taken at end of chill; serum yellowish. One-tenth cubic centimeter defibrinated blood added to each tube containing 1 cc. of bile-quinine mixture. Tubes incubated one hour at 37°C., then left at room temperature ten hours.

Bile (1:50), cc..........................	1.0	1.0	1.0	1.0	0.0	0.0
Quinine mgm......................	0.2	0.3	0.4	0.0	2.0	0.0
Results, (per cent of cells laked)....	20	30	35	4.0	Trace	0.0

Experiment 4. Effect on blood of blackwater fever immediately after fresh attack of malaria. Blackwater fever January 23. Malaria March 1. Tests March 2. Procedure same as in experiment 3.

Bile (1:75), cc...	1.0	1.0	1.0	0.0	0.0	0.0
Quinine, mgm....	0.2	0.3	0.0	2.0	0.3	0.0
Malaria blood...	70 per cent hemolysis	Complete hemolysis	Trace	50 per cent hemolysis	No hemolysis	No hemolysis
Normal blood....	Trace	20 per cent hemolysis	Trace	No hemolysis	No hemolysis	No hemolysis

Experiment 5. Effect of quinine-bile mixture on normal blood. Procedure same as above.

BILE (1:50)	QUININE	BLOOD A		BLOOD B	
		Twenty-four hours	Forty-eight hours	Twenty-four hours	Forty-eight hours
cc.	*mgm.*				
1.0	0.2	±	+	±	+
1.0	0.3	+	+	±	+
1.0	0.4	+	+	+	++
1.0	0.0	−	Trace	−	Trace
0.0	0.4	−	−	−	−

Experiment 6. Effect of bile-quinine mixture on rabbit blood. Procedure as above.

					REMARKS
Bile (1:5)............	0.10	0.15	0.15	0.1	The volume was made up to 1 cc. Tubes left two hours at 37°C., then at room temperature
Quinine, mgm........	0.2	0.2	0.0	0.0	
Results four hours....	−	+	−	−	
Results twenty-four hours..............	±	++	±	Trace	

Experiment 7. Effect of different kinds of biles. It became evident in the course of the experiments that different samples of bile varied in their effect. The following is a typical experiment. The procedure of the test is the same as above.

	BILE (1:50)	QUININE			REMARKS
		0.2 mgm.	0.3 mgm.	0 mgm.	
	cc.				
A (dark green)	1	80 per cent hemolysis	Complete hemolysis	Trace	One hour at 37°C., then at room temperature
B (yellowish green)	1	30 per cent hemolysis	50 per cent hemolysis	0.0	

The experiments cited above demonstrate hemolysis by combinations of bile and quinine solutions that are ineffective separately. Neither the bile salts nor the reaction of the bile seem to be the factors concerned. The tests were always made with solutions of neutral reaction, while the bile salts themselves did not give the effects of the whole bile.

EXPERIMENTAL HEMOGLOBINURIA

A series of attempts was made to produce hemoglobinuria in rabbits by the injection of bile and quinine. In some experiments varying doses of quinine were injected on a series of successive days, followed by a moderate dose of bile. In others the bile was injected first and then the quinine. In still others mixtures of the two substances were injected.

In one experiment only were definitely positive results obtained; in four others the results were doubtful and in the larger number of experiments the results were negative.

In the successful experiment a typical picture of hemoglobinuria was obtained.

Four cubic centimeter sterile ox bile was injected intravenously into a rabbit weighing 1100 grams. Five minutes later 30 mgm. of quinine hydrochloride was given by the same route. Before the injection, the serum was colorless. After the injection of the bile there was marked hemoglobinemia—such as can be obtained by laking 0.01 cc. of rabbit red cells in a fluid bulk—which diminished rapidly, so that two hours after the injection the serum was only slightly tinged with red. One hour after the injection of the quinine a small quantity of urine was obtained which contained a fairly large amount of hemoglobin, during the next twenty-four hours only 8 cc. of urine being voided. This urine still contained hemoglobin but quinine was absent. The next day hemoglobin was absent but quinine was present.

The control animal receiving bile alone died immediately after the injection, while the one receiving quinine alone showed no effect whatever despite the proportionately large dose administered.

The doubtful results were all obtained by the injections of 40 mgm. of quinine on two succeeding days followed by 1 or 2 cc. of bile. In all the four experiments the urine collected during the twenty-four hours following the injection of bile contained traces of hemoglobin and albumin. The results were not, however, conclusive and could not be reobtained at will.

DISCUSSION

The series of observations reported in this paper seem to have a bearing on the mechanism of quinine hemoglobinuria. It appears that in the presence of bile quinine becomes highly hemolytic even in the small doses normally employed in the treatment of malaria. Some as yet undetermined constituent of bile has the power of rendering quinine hemolytic in one-tenth the concentration usually required. If we assume that the substance in question is liberated during the active destruction of blood cells in the course of repeated attacks of malaria, a simple theory of the mechanism of hemoglobinuria suggests itself. Hemoglobinuria patients invariably give a history of repeated attacks of malaria. In the course of these attacks cell products are constantly liberated in the circulation which may not at times be readily eliminated and thus reach a concentration higher than normal. At that time the injection of quinine causes hemolysis and hemoglobinemia which manifests itself by the appearance of hemoglobin in the urine.

Experiments on rabbits carried on thus far have not yielded satisfactory results because of the difficulty of reproducing the exact conditions developed by chance in human beings. Hemoglobinuria was produced by the injection of bile but the dose injected bordered on lethality and consequently could not be relied on to give constant results. In four experiments mild hemoglobinuria was obtained by the injection of quinine followed by bile.

It is realized that the observations reported are of a fragmentary character. They are, however, of value in so far as they establish that quinine becomes actively hemolytic even in small doses when acting in combination with bile, a product of destruction of red blood cells.

The experiments are being continued with a view to finding some specific product of blood cell destruction which will increase the hemolytic power of quinine in the same manner as bile and the conditions required to produce hemoglobinuria experimentally in animals.

RESUMEN

La serie de observaciones relatadas en este artículo parecen tener cierta relación con el mecanismo de la hemoglobinuria de quinina. En presencia de bilis, la quinina se manifiesta altamente hemolítica aún en las pequeñas dósis comúnmente empleadas en el tratamiento de la malaria. Algún constituyente indeterminado de la bilis tiene el poder de hacer a la quinina hemolítica en una décima de la concentración usualmente requerida. Si asumimos que la substancia en cuestión se descarga durante la destrucción activa de las células de la sangre, después de repetidos ataques de malaria, esto en sí sugeriría una sencilla teoría del mecanismo de la hemoglobinuria. Los pacientes de hemoglobinuria invariablemente presentan una historia de repetidas recidivas maláricas. En el curso de estos ataques productos celulares son constantemente descargados en la circulación, que al no ser eliminados, alcanzan concentración abnormal. A veces la inyección de quinina causa hemólisis y hemoglobinuria que se manifiesta con la aparición de hemoglobina en la orina.

Los experimentos con conejos llevados a cabo no han dado resultados satisfactorios debido a la dificultad de reproducir las condiciones exactas que en oportunidades se desarrollan en el humano. Se produjo hemoglobinuria mediante la inyección de

bilis pero la dosis administrada se acercaba tanto a la letalidad que en consecuencia no se confía que dé resultados constantes. En cuatro experimentos se obtuvo hemoglobinuria benigna mediante inyección de quinina seguida de bilis.

Se comprende que las observaciones relatadas son de caracter fragmentario. Su valor consiste, sin embargo, en que establecen que la quinina se hace altamente hemolítica aun en pequeñas dosis en combinación con la bilis.

Se continúan estos experimentos con el propósito de averiguar si existe algun producto específico en la destrucción de las células rojas que aumente el poder hemolítico de la quinina, de igual manera que la bilis, y bajo las condiciones requeridas para producir la hemoglobinuria experimentalmente en animales.

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ACETIC-ALCOHOL FIXATION FOR PARASITIC AMOEBAE AND FOR TISSUES

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STAINING THE PARASITIC AMOEBAE

Carnoy's acetic-alcohol has been found to be an excellent agent for fixation of the parasitic amoebae, embracing, as it does, almost instantaneous killing with rapid fixation and penetration without the aid of heat, which latter so often caused misleading appearances through the shrinking of tissue elements. Acetic-alcohol is rapid, three hours sufficing for even large pieces of tissue, while from ten to fifteen minutes is ample for any of the smear preparations. It also is an excellent fixative preceding any of the hematoxylin stains.

Fidelity of the nuclear structure is better held, as shown by the accompanying photomicrographs of preparations prepared by this method than those prepared with the Schaudinn hot fixative. The acetic acid in the Carnoy fluid hemolyzes the erythrocytes, which at times is of value as leaving the internal structures of the parasite even plainer in detail than is possible with those methods where the red cells are fixed and take on an intense stain as generally occurs.

The method of fixation and staining follows:

Smears are made on cover-glass or slides and before they can dry are immersed in freshly prepared Carnoy's acetic-alcohol. At no stage of the process of fixation or staining must the preparation be allowed to become dry.

1. Fix in Carnoy's acetic-alcohol for ten to fifteen minutes. Care must be exercised to prevent overfixing and with average smears ten minutes will be ample time for complete fixation.

Prepare the fixative as follows:

Absolute alcohol	6 parts
Chloroform	3 parts
Glacial acetic acid	1 part

2. Wash in one change of absolute alcohol.

3. Absolute alcohol ten to twenty minutes.

4. Ninety-five per cent alcohol ten to twenty minutes.

5. Distilled water fifteen to twenty minutes.

6. Three and five-tenths per cent solution of iron-alum in water from thirty minutes to over night, the latter preferably.

7. Wash rapidly in distilled water.

8. Stain in the following: (Rosenbush hematoxylin)

(*a*) Saturated aqueous solution of lithium carbonate.

(*b*) One per cent hematoxylin in 95 per cent alcohol.

Just before using, add solution (*a*) to solution (*b*) until the mixture is a cherry red, 4 or 5 drops of the lithium solution to 10 cc. of the hematoxylin generally being sufficient. Stain twenty to thirty minutes to over night. Thirty minutes has proven satisfactory with the freshly prepared stain. Some authorities state that the hematoxylin solution should be allowed to ripen ten days to two weeks, but actual experience has shown the freshly prepared hematoxylin solution ripened with a little more of the lithium solution to be more satisfactory and certain in results.

9. Wash thoroughly in distilled water.

10. Differentiate with a weak iron-alum solution (3 parts of distilled water to one of the iron-alum is satisfactory), until the nuclear structure shows clearly; the examination is made in water under a cover-glass.

11. When the differentiation is completed the slide is rapidly washed in distilled water, passed through 95 per cent alcohol, absolute alcohol and xylol to xylol balsam for mounting.

The stain is permanent.

With suitable material it is believed that the use of Carnoy's acetic-alcohol fixative also will prove of value in staining tissue for the purpose of showing the protozoan parasites in situ. Here another favorable feature of this fixative is noted when it is seen that the tissue is carried from the fixative to absolute alcohol, to chloroform, chloroform-paraffin and paraffin, all within twenty-four hours.

SUMMARY

The use of Carnoy's acetic-alcohol as a fixing agent affords a rapid and certain method of fixing the parasitic amoebae for staining. This agent, consisting of absolute alcohol 6 parts, chloroform 3 parts, and glacial acetic acid 1 part, is used cold and insures quick killing, rapid fixation and fidelity of tissue elements when followed by hematoxylin stains. Fresh smears are completely fixed in ten to fifteen minutes and pieces of tissue in three hours. The fixative is followed by washing in absolute alcohol (one change) following which the tissue or smear is placed in absolute alcohol.

ACETIC-ALCOHOL FOR TISSUE FIXATION

For the rapid preparation of paraffin sections Carnoy's acetic-alcohol fixative offers a certain means of fixation and good staining in from eight to sixteen hours. The tissue so prepared shows the nuclear elements even better than that prepared by the ordinary methods which take from four to ten days, and will often permit of the use of paraffin in emergency work where other methods are impracticable, making the study of the tissue difficult, if not entirely unsatisfactory on account of shrinkage and tissue changes caused by heat, or the stronger fixatives ordinarily used in the quicker methods of preparation. Paraffin as an embedding agent certainly gives better sections than is to be expected from the several methods of preparing frozen sections for emergency work.

The method of use follows:

1. Fix small pieces of tissue in Carnoy's acetic-alcohol (absolute alcohol 6 parts, chloroform 3 parts, glacial acetic acid 1 part; must be freshly prepared) for one to two hours.
2. Wash in absolute alcohol, one change.
3. Absolute alcohol for one to two hours.

4. Chloroform after blotting off alcohol for one and one-half hours.
5. Chloroform-paraffin for from two hours to over night.
6. Paraffin for one to two hours.
7. Embed.

After cutting, the sections are affixed to the slide with an albumen water prepared from the white of an egg (25 cc. of the egg white whipped with 15 cc. of water, to which a crystal of thymol is added to prevent the growth of fungi-glycerin is not used) and the section fixed to the slide with the aid of sufficient heat to just start the paraffin melting. Start through the stains at once.

Experience has shown that it is sufficient to use one change of xylol for five to eight minutes, one change of absolute alcohol for three to four minutes; and one change of 95 per cent alcohol for three to four minutes, in bringing the sections into water for staining. Likewise no alcohol lower than 95 per cent is used for the dehydration preparatory to mounting in balsam, one change of 95 per cent and two changes of absolute alcohol and two changes of xylol being used for dehydration and clearing, the last change of xylol being allowed to act for from five to ten minutes for the final clearing.

Acetic-alcohol is an excellent fixative for almost any of the staining methods, specially hematoxylin. In this laboratory Harris hematoxylin and eosin are routinely used.

I am indebted to Major R. E. Scott, Medical Corps, United States Army, in charge of this Laboratory, for supervision and approval of this work, and to Major J. F. Coupal of the Army Medical Museum for the photographs.

SUMARIO

El alcohol-acético de Carnoy es un agente de fijación rápido y cierto en el teñido de las amebas parasíticas. Consiste de 6 partes de alcohol absoluto, 3 partes cloroformo, y una parte de ácido acético glacial. Se usa frío y se procede seguidamente

a la coloración con los tintes de hematoxilina. Frotes frescos se fijan dentro de 10 a 15 minutos y secciones de tejido en tres horas. Tan pronto termine la fijación se procede a un lavado en alcohol absoluto y seguido se coloca el frote o el tejido en alcohol absoluto.

EXPLANATION OF PLATES

PLATE 1

Fig. 1. Endomebae stained following fixation with Carnoy's acetic-alcohol. The vacuolization resulting from the acetic acid hemolyzing the erythrocytes is noticeable.

Fig. 2. Endomebae stained following the usual Schaudinn fixative.

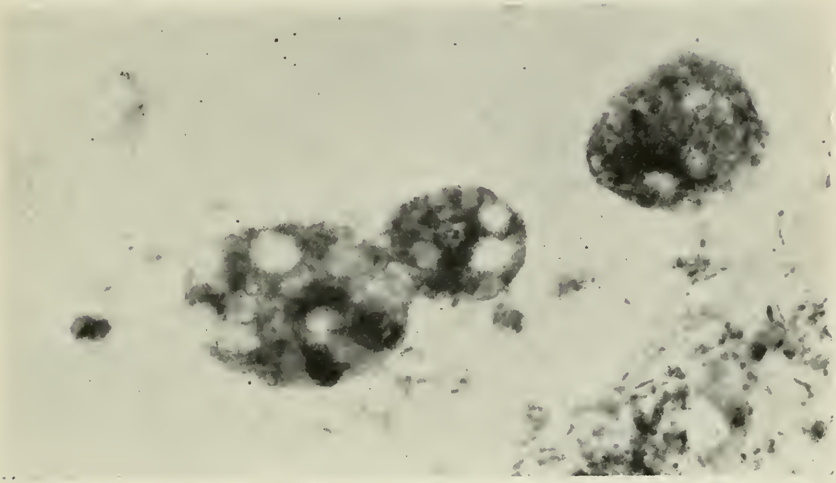

FIG. 1

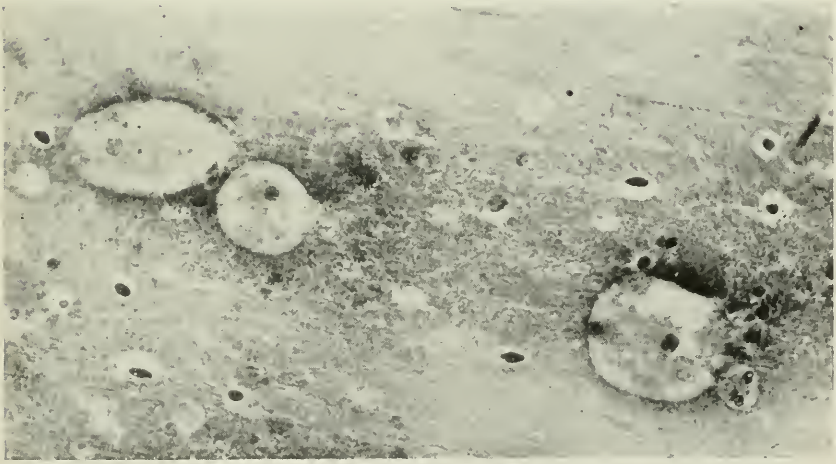

FIG. 2

PLATE 2

FIG. 3. Photo-micrograph of endomebae following Carnoy fixation showing details apparent with a higher magnification.

FIG. 4. Endomebae stained following Schaudinn fixative at a corresponding magnification.

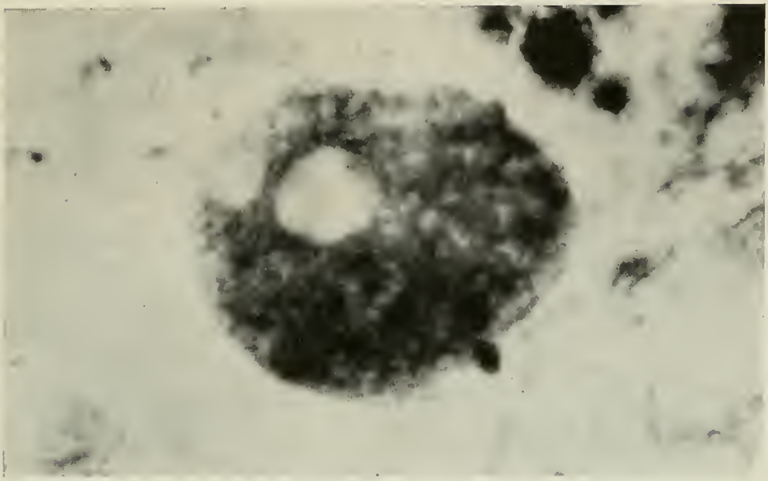

Fig. 3

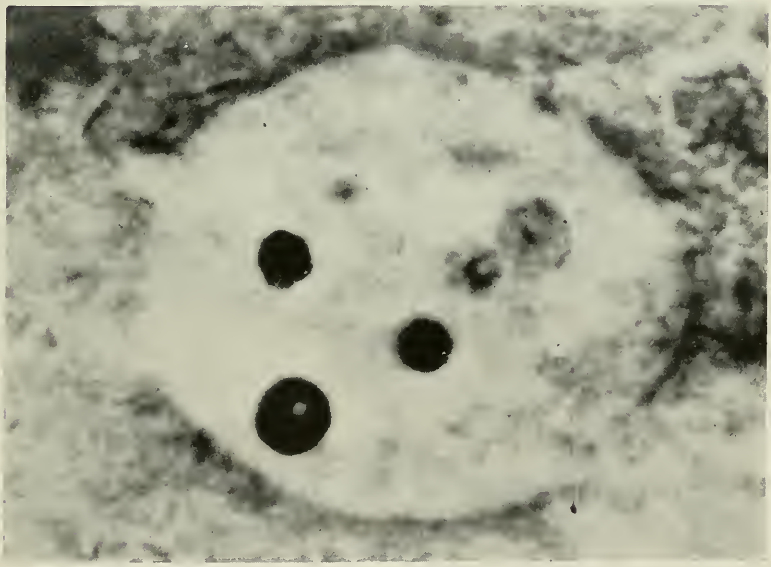

Fig. 4

PLATE 3

Fig. 5. Endomebae after Carnoy fixation, showing apparent vacuolization resulting from the hemolysis of the erythrocytes by the acetic acid of the fixative. Higher magnification.

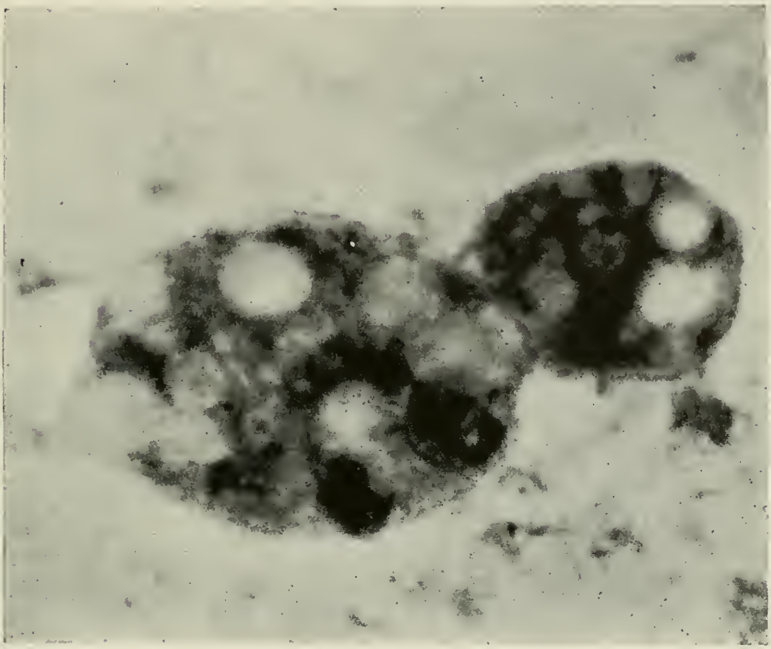

FIG. 5

THE MOSCOW TROPICAL INSTITUTE[1]

(*Kudrinskaya Place, No. 8, Moscow, Russia*)

SH. MOSHKOVSKY

Received for publication March 9, 1923

Malaria and tropical diseases were always widespread in Russia. In connection with war, the mass movements of troops and groups of workers, as well as with the extraordinarily hot temperature of recent summers, malarial diseases increased very much even in European Russia, and mass attacks of malaria occurred even in the north as far as Archangel. Besides marlaria there are also other protozoan diseases very widely spread in Russia, expecially in the Caucasus and Turkestan. These include Kala Azar, leishmaniosis of the skin, Bouton d'orient, Pappataci and dengue fever, ameobic dysentery, colics caused by *Balantidium coli* and *Lamblia*, etc. There is also considerable economic damage caused by various protozoan diseases of animals, such as horse piroplasmosis, trypanosomiases, etc.

The task of the Tropical Institute (opened September 1, 1920) is the study and combating of the above diseases.

Professor E. J. Martsinovsky was appointed director of the institute.

The activities of the institute are as follows:

1. Work of a purely scientific character—study of parasitology, epidemiology and clinic of diseases of hot countries. For this purpose the institute also organizes systematic scientific expeditions to various regions of the Caucusus, Turkestan, etc.
2. Scientific pedagogical work—preparation of physicians for the special combating of the above diseases, leaders of malaria stations, etc. Each year continuation-courses for physicians are are organized in the institute.

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[1] Translated and transmitted by H. J. Muller, Associate Professor of Zoology, University of Texas, Austin, Texas.

3. Publicity work—popularization of methods for the fighting of tropical diseases.

4. Consultative work and the rendering of aid in various operations for the realization of practical measures in fighting the above diseases.

The divisions of the institute are:

1. Protozoological division—leader, Prof. E. J. Martsinovsky.
2. Chemotherapeutical division—leader, Prof. J. Smorodintsev.
3. Entomological division—leader, Prof. W. Nikolsky.
4. Helminthological division—leader, Prof. K. Skryabin.
5. Clinical division—leader, Dr. W. Nosina.

At present the scientific work in the institute is proceeding in many directions, as follows:

Prof. E. Martsinovsky: Leishmanioses; recurrens (relapsing fever); a new septic paratyphoid-like disease.

Dr. Sh. Moshkovsky; Cultures of *Leishmania tropica* and various trypanosomes; chemistry of histological staining; and chemotherapy of malaria.

Dr. N. Shakov and Dr. Isayev: Culture of malaria plasmodia. Practical combating of malaria. (The latter has gone to Buchara for this purpose.)

Dr. W. Nosina and Dr. O. Sutshkova: Clinic of malaria and other tropical diseases, such as Kala Azar, etc.

Dr. W. Povshina and A. Shtchurenkova: Experimental oriental tumors; *Trichomonas vaginalis* and its rôle in pathology. (At present they are taking part in a scientific expedition in the Caucasus.)

Prof. J. Smorodintsev and Dr. A. Adova et al.: The action of quinine upon enzymes. The enzymes of the insect-catching plant, *Utricula vulgaris,* which destroys mosquito larvae.

Prof. K. Skryabin and Dr. P. Popov: Helminthology. (The latter also works in the entomological division.)

Dr. M. Kanevskaya: Etiology and clinic of *Pyorrhoea alveolaris.*

Dr. A. Metielkin: Protozoan diseases of animals.

The institute also possesses a museum, in the four halls of which a considerable number of preparations, illustrations, diagrams, models, etc., are assembled. Studios for micro-photocinematography, and for the preparation of models are also installed at the institute.

At certain times scientific conferences take place in the Tropical Institute. Only short references may be made here to some of the proceedings of those conferences which were held in the first half of the year 1922:

January 17, 1922. Dr. Sh. Moshkovsky: On the mechanism of the action of quinine in malaria. On the basis of an analysis of observations of various authors and the experiences of the clinic of the Moscow Tropical Institute the speaker claimed that the action of quinine is indirect, and inclined towards the point of view of J. Morgenroth and G. Cremonese. The anti-malarial action of quinine is perhaps dependent for the most part on its storage in the erythrocytes. The merozoids and sporozoids can not penetrate into the quinine-laden erythrocytes and they founder in the blood plasma. Mühlen's experiments, and also the lack of truly quinine-resistant races of Plasmodium speak against the binding of quinine by the Plasmodia. The binding of quinine by the erythrocytes can also be seen in connection with the pathogenesis of blackwater ("Schwarzwasser") fever. (The first case in Moscow occurred in the clinic of the Tropical Institute.) Perhaps this storage of quinine in the erythrocytes may also be of great significance in the antipyretic action of quinine.

January 24, 1922. Prof. E. Martsinovsky: On oriental tumors and their exciting causes. After a short presentation of the present status of the problem the speaker demonstrated a tumor which the co-worker of the institute, Dr. A. Shtchurenkova, had removed for scientific purposes during an expedition to Turkestan. Cultures of *Leishmania tropica* were also demonstrated, which she had succeeded in cultivating from this tumor. Systematic cultivation of *Leishmania tropica* was later undertaken by Dr. Sh. Moshkovsky, who on February 14 bred a Leishman culture from a tumor upon N. N. N. agar and later carried it through many more generations in vitro. The cultures have until now (August 24, 1922) been entirely similar to the first one. In old (one and one-half to four months) cultures of *Leishmania tropica*, round and motionless, non-flagellate, deeply-staining forms were observed by Dr. Moshkovsky, which contained only a nucleus and a blepharoplast, and which, upon transference into a new tube, again changed over into typical flagellated forms with long flagellae. The cultures of *Leishmania tropica* also succeeded on plates of blood-agar.

January 24, 1922. Dr. M. Kanevskaya: On some methods of staining malaria plasmodia in connection with the problem of the nature of the Romanovsky staining process. Speaker gave a digest of various results of his work on the nature of the Romanovsky-Giemsa stain. The Romanovsky effect, as is well-known, consists of a red coloration of several elements of the preparation (nuclei, azurophil granules, etc.) after staining with mixtures of azure containing methylene blue and eosine. In this effect the eosine plays the rôle of a dye and in no case of a mordant, as Unna has assumed. The attempts to displace eosine with its chemical components or with other substances (Nocht, Unna) do not give a real Romanovsky effect, because a characteristic bright red or bright red-violet shade is never attained thereby. The effects of Neumann and Scott too are not identical with the Romanovsky one. On the basis of the study of the chemical constitution of the methylene azure the speaker came to the conclusion that the methylene azure plays the rôle of an amboceptor, which unites through its S-atom with the acid protein of the substrate, and in addition through its free amid group with the eosine. An easy method of rapid staining of malaria plasmodia was also presented, which gives a pseudo-Romanovsky effect: the nuclei of the schizonts take on a red color but not those of the gametocytes. The procedure is as follows: stain one-half minute with alkaline methylene blue solution (e.g., diluted Mauson solution), rinse, then immerse (for an instant only) in 5 to 10 per cent tannin solution, and again rinse. The light blue protoplasm of the plasmodia stands out very conspicuously against the rose-violet erythrocytes. The pigment and the Schüffner granules also appear plainly. The leucocytes too are beautifully colored.

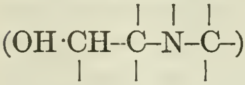
February 14, 1922. Prof. K. Skryabin: A short review of the new American text books of medical helminthology. The helminthological part of the text book of Fantham, Theobald and Stephens is very much antiquated: it has been for the most part written after Braun, 1908. There are also many chronological mistakes and errors of fact. Still, this book is much more acceptable than the book of D. Rivas, *Human Parasitology,* which contains many errors and unsuccessful (*"ungelungene"*) tables of classification and in a number of respects has remained forty to forty-five years behind the times.

Dr. P. Popov: Rats and mice as enemies of mankind and the means of combatting them. An exposition of the economic damage which rats and mice occasion, and their rôle in the spread of plague and many other diseases. The enormous increase of rats in Russian cities during recent years was calculated and represented as a very serious pest.

February 21, 1922. Prof. E. Martsinovsky: A new septic disease similar to Recurrens. In a number of cases of typhoid-like infections bipolar stainable rodlets were found included within the leucocytes. The sickness begins with a neurotic angina. The spleen is much enlarged. It proceeds to the *exitus lethalis* with the appearance of symptoms of heart weakening. In the cases in which recovery occurs the illness lasts ten to twenty days. Pathological-anatomical finding: much swollen spleen with small infarcts; in the kidneys nidiform (*"nestige"*) infarcts with the bipolar bacilli; focal hepatitis with the same rodlets included in the leucocytes and in Kupfer's "star-cells." Successful attempts were made to breed a bacterium in pure culture from the blood of living patients and from the spleen and blood of the heart of patients deceased. This microörganism is weakly motile, Gram-negative, does not liquify the gelatin, splits glucose, with attendant formation of gas, but not lactose, does not affect milk, is not agglutinated by typhoid and parathyphoid A, B serum, and is very pathogenic for laboratory animals.

March 7, 1922. Dr. Sh. Moshkovsky: Towards the pathogenesis and therapy of scurvy. Review of the rôle of the gut in the causation of scurvy, and its condition at the height of the disease. As scurvy is an "avitaminosis," it is possible specifically to influence the causation of the disease only by means of a diet rich in factor C. Brewer's yeast, which lacks this factor C, can act only as a secondary aid, when, by virtue of its content of factor B, it exerts a favorable action upon the nervous system and gut.

April 4, 1922. Dr. M. Kanevskaya: On the etiology and chemotherapy of Pyorrhoea alveolaris. The problem of the exciting cause is not yet solved. In the case of the patients dealt with various spirochaetes and *Entamoeba buccalis* were found in the mouth; the same microörganisms are also found in healthy subjects however. Good results have followed the employment of neosalvarsan: smearing of the gums and intravenously. Subcutaneous emetin injections produce a lessening of the number of Amoebae.

April 25, 1922. Prof. J. Smorodintsev: The action of quinine preparations on the enzymatic functions of the organism: I. The connection between the chemical constitution and the pharmacological action of quinine. The vinil-, methoxyl-, and secondary alcohol groups serve as the active groups of the quinine molecule. The specific action of quinine depends upon the atom group which unites the quinolin remainder with the "chinuklidin" nucleus  $(\mathrm{OH}\cdot\underset{|}{\mathrm{CH}}-\overset{|}{\underset{|}{\mathrm{C}}}-\overset{|}{\mathrm{N}}-\overset{|}{\underset{|}{\mathrm{C}}}-)$, whereby the anti-malarial characteristics of quinine are dependent upon the di-piperidin nucleus, but the antipyretic properties upon the "chinuklidin" nucleus. Experimental investigations showed that quinine exerts practically no influence upon the action of reducases.

Dr. A. Adova: The action of quinine upon α-proteases. Quinine hydrochloricum and sulphuricum hinder the digestion of egg white by the pancreatic juice. The double union of quinine hydrochloride with urea hastens this process; the chlorate, sulphate, and nitrate of urea act in like manner; free urea, however, has no effect.

May 23, 1922. Prof. A. Kissel: On the quinine therapy of malaria. A method of treatment was described, which the speaker had carried thru in 133 cases. A full dose, 1.0 *pro die*, is divided into two half-doses, and given in this manner (one half dose twice a day) each day for three months; then one half-dose is given only once daily for three months; then, after a six weeks pause, one half dose once daily for six weeks again; then again a six weeks pause, followed by another six weeks of one half dose once daily. No unpleasant consequences of such prolonged quinine medication were observed. The speaker was well satisfied with the results of his therapy.

OBSERVATIONS ON THE ETIOLOGY OF DENGUE FEVER

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I. INTRODUCTION

During the summer and fall of 1922, accompanying a scourge of yellow fever mosquitoes (*Aëdes aegypti* [= *Stegomyia fasciata*]) such as has not before been recorded, an epidemic of dengue fever of unprecedented proportions in the United States, if not in the world, occurred in Texas and neighboring states. The epidemic originated in Galveston during the second week of June, and raged there for a number of weeks before spreading elsewhere. In July one of us (Rice, 1922) sent for publication a note on the epidemic as a warning to the health officers in other parts of the State to be on the lookout for an outbreak of the disease. The first cases reported to the City Health Department at Houston occurred in the closing days of July, and by the end of the first week in August the cases became numerous. From Houston the disease spread along the railroad routes to all parts of east and central Texas, and to the neighboring states. The number of cases in the State of Texas has been estimated by the State Board of Health at between 500,000 and 600,000 cases. In Galveston there were probably not less than 30,000 cases; in Houston, 30,000; in Dallas, 20,000, etc. The average cost per case for medical attention and drugs can be conservatively estimated at \$3.00; in addition to this there was an average loss of time of five days, followed by five days of inefficiency, amounting to at least seven and one-half days of lost time. For the entire State the loss from the epidemic, therefore, can be estimated at not less than $1,500,000, plus 3,750,000 lost days, which in the case of professional and business men, students, etc., was a much more serious loss than the direct financial cost. The clinical aspects of the disease as it appeared in this epidemic have been described in a previous paper by one of us (Rice, 1923) and also by Scott (1923).

The unusual opportunities afforded by the epidemic for etiological investigation were fully appreciated, but unfortunately could not be taken advantage of to the extent desired. One of us (L. R.) was on the ground in Galveston during the entire epidemic, but in the absence of colleagues to assist in the work at the University of Texas Medical School, and on account of the demands on time made by hundreds of patients, was unable to do any experimental work before the end of July, and only a limited amount after that. One of us (A. C. C.) did not arrive on the scene until the latter part of September, and was then distracted from continuous work by the duties incident to the beginning of a new school year. By the time our work became organized and arranged in a coöperative manner, the epidemic began to abate and had practically ceased before we were able to carry out a number of experiments which had been planned. It is hoped that some of this work may be carried out at a later date.

II. ATTEMPTS TO DETERMINE CAUSATIVE ORGANISM

Historical résumé

Numerous attempts have been made to determine the causative organism of dengue, but, although a number of supposed parasites have been found, no organism which can be considered as having an etiological relation to the disease has yet been discovered and verified. A good review of the earlier work along this line was given by Ashburn and Craig (1907), who showed that the organism was a filtrable virus. Cleland Bradley and MacDonald (1918, 1919) showed that the organism was present in serum and in citrated blood, and would in some cases adhere to corpuscles even after repeated washing. They further showed that the virus remained infective in blood outside the body at room temperature for four days, and in an ice chest up to seven days.

The discovery by Noguchi of *Leptospira icteroides* in yellow fever, which is in all probability the causative organism, has led to a very general opinion that by similar methods a Leptospira would probably be demonstrated in dengue blood. Couvy (1921) reports finding delicate spirochetes in the blood immediately before the onset of the disease, but not during the fever; no others have reported Leptospira-like organisms in dengue blood although search has been made for them by many competent observers in various parts of the world. There is a persistent belief, repeatedly expressed by scientific writers, that dengue and yellow fever are related diseases, this opinion being based largely on the epidemiology of the diseases, but partly also on the occasional occurrence, in dengue, of characteristic yellow fever symptoms, e.g., jaundice and "black vomit." A leucopenia, sudden onset, and occasionally slow pulse in dengue are also reminiscent of yellow fever, and the present knowledge of the transmission by mosquitoes, especially since the evidence is becoming overwhelming that the same species of mosquito is involved in both diseases, tend still further to indicate a relationship. On the other hand, in our opinion, the similarity between the two diseases appears to have been overemphasized. The outstanding features of yellow fever are jaundice, albuminuria and relatively slow pulse. Neither jaundice nor albuminuria can be considered diagnostic in dengue as one of us (Rice, 1923) has pointed out, and the pulse is most commonly proportionate to the temperature. Furthermore, the rash, which is one of the most characteristic features of dengue, does not occur in yellow fever. The lay opinion that dengue is in some way related to yellow fever or, as frequently expressed, "a mild form of yellow fever" seems to be traceable to unguarded statements by physicians, either to patients or in the public press.

Examination of blood

In view of the prevailing opinion, however, that dengue would be found to be caused by a Leptospira, we determined to make every effort to find such an organism if it were present. In all we examined about 250 slides of fresh blood from about 70 patients, and on various days of the disease, with dark field microscopes, and failed to find any Leptospira or other spirochete-like organism. Numerous stained slides were made and examined, but nothing which was suggestive of an organism was found in either plasma or cells. In a few cases fresh urine was centrifuged and examined under a dark field, but with negative results.

Animal inoculations

Animal inoculations were tried with guinea-pigs, white mice, and a rhesus monkey. Fourteen guinea-pigs were inoculated intraperitoneally with from 2 to 3 cc. of whole blood immediately after it was drawn from the patient, 2 guinea-pigs being inoculated simultaneously from each of 7 patients, the blood being taken on various days of the disease from the first to the fifth. In all cases but 1 the guinea-pigs remained perfectly well during the period of observation (three to four weeks), and showed no significant rise in temperature. One guinea-pig (no. 3) inoculated from a patient twenty-four hours after onset, appeared slightly sluggish and indisposed on the fifth day, with a temperature of 102.2°F., which, however, he had registered before inoculation. This pig was killed and autopsied, and 2 other pigs inoculated intraperitoneally with its blood, one with 4 cc., the other with 2 cc. Neither of these pigs showed any signs of illness, or any lesions, nor were any pathological conditions found in pig 3. Inasmuch as Noguchi (1919) found that yellow fever did not always produce a typical disease in guinea-pigs until the first or second subinoculation, we drew blood from the heart of another guinea-pig (no. 6) on the fifth day after inoculation with a patient's blood taken on the second day of the disease, and inoculated 1 cc. intraperitoneally into pigs 19 and 20. On the fifth day we drew heart's blood from pig 19 and inoculated it in like manner into pigs 17 and 18, but in all cases with negative results. The blood from pigs 3, 6, 18 and 19 was examined under a dark field microscope, but with negative results.

From another patient 2 guinea-pigs were inoculated in a somewhat different manner. One cubic centimeter of a patient's blood, drawn on the second day of the disease, was diluted with 1 cc. of heart's blood from the guinea-pig by immediately plunging the needle containing the patient's blood into the pig's heart and withdrawing enough blood to make 2 cc. In one of these pigs (unanesthetized) 1 cc. of the mixture was re-injected into the heart, the remaining 1 cc. being inoculated intraperitoneally. In the other pig, which was under ether anesthesia, the same procedure was followed except that the intraperitoneal injection was not made, the second 1 cc. of blood being discarded. Both of these pigs remained well, but thirty-one days later 6 cc. of blood was withdrawn from each of them to lower their vitality (Japanese investigators found that guinea-pigs succumbed in from seven to thirty-six days), but this had no effect.

It occurred to us that it might be possible to produce infection in guinea-pigs by using a very small quantity of blood, thus carrying over fewer antibodies, if these were present. Noguchi (1919) apparently found that such antibodies were a factor in animal inoculations and it was evident that very small quantities of blood were sufficient to cause infection. Koizumi, Yamaguchi and Tonomura (1917) state that 0.00005 mil. was found to be infective for man. To test this point we withdrew 0.1 cc. of blood from a patient in the second day of the disease and diluted it with a guinea-pig's heart's blood to 1 cc. and then re-injected 0.02 cc. of the mixture into the heart, thus inoculating 0.002 cc. of patient's blood if the mixture had been perfect, but in all probability the pig received considerably less than this of the patient's blood. This pig also remained well. Furthermore, 65 guinea-pigs, of all ages, were housed in an outdoor pen during the entire summer and fall, where yellow fever mosquitoes swarmed. Only one became ill, and three days later died from a respiratory infection. These pigs were freely bitten, even tormented, by the mosquitoes, and the percentage of dengue in that district was high.

These results with guinea-pigs are in harmony with results obtained by Cleland, Bradley and MacDonald (1919) in Australia, who inoculated a non-immune human being with 2 cc. of blood drawn from a guinea-pig on the seventh day after inoculation with dengue blood, with negative results. There is, however, no corroboration of the work of Koizumi, Yamaguchi and Tonomura (1917) who state that small amounts of defibrinated dengue blood were lethal to guinea-pigs in from seven to thirty-six days, and that blood taken from pigs inoculated fifteen days previously was lethal to other pigs in from five to nineteen days when inoculated intraperitoneally or subcutaneously, and in from twenty-eight to thirty-four days if inoculated intravenously, while the third transfers were negative. It is significant that in an earlier paper Yamaguchi, Ditsumi and Tonomura (1916) state that inoculations into guinea-pigs were without results. We conclude, therefore, that guinea-pigs are not susceptible to dengue organisms.

We inoculated 6 white mice with about 0.5 cc. each of fresh citrated dengue blood taken on the third day of the disease. With the exception of one mouse which died during the inoculation all of the mice remained perfectly well. Rats were not available for experimental work.

We inoculated a young rhesus monkey with about 5 cc. of citrated dengue blood, drawn on the third day of the disease, about two hours after the blood was taken. Although the monkey, according to his keeper, appeared to be chilly and unusually morose on the third and fourth days after inoculation, he showed no significant rise in temperature. On the fifth day he showed a fine papular rash on the chin and throat, which, however, we do not think was a manifestation of dengue. No other symptoms were observed and we are inclined to think that the inoculation had negative results, as did those of Lavinder and Francis (1914); at any rate, it was very doubtfully positive.

Although no experiments were made on cats or dogs, it is inconceivable that great numbers of these animals were not bitten by infected mosquitoes in such cities as Galveston and Houston, where a very high percentage of human beings were infected;

yet careful inquiry from veterinarians and from families in which dengue had occurred, and in whose houses dogs and cats were kept, gave no evidence that any indisposition had been shown by these animals.

Culture methods

Our greatest reliance in finding a Leptospira, if one were present, was placed in cultural methods. We did not use any of the ordinary bacterial culture media, since these had been tried unsuccessfully by others. We did, however, make a considerable number of cultures of dengue blood in a medium which Noguchi found successful in the cultivation of *Leptospira icterohemorrhagiae* and of *Leptospira icteroides*. Two kinds of semi-solid culture media were prepared; one contained 3 parts of Ringer's solution, 1 part of citrated plasma, and 1 part of 1.5 per cent plain agar, giving the final medium 0.3 per cent agar; the other contained 4 parts of Ringer's solution and 1 part of citrated plasma. Ten-cubic centimeter tubes of the agar medium were inoculated with 1 cc. of patient's whole blood, while 10-cc. tubes of the Ringer-plasma medium were inoculated with 2 cc. of patient's whole blood, to give a loose fibrin network. In all cases care was taken to use blood from individuals who had never had an attack of dengue, and the blood of several different individuals were used to avoid, if possible, the chance of obtaining a naturally immune blood which might be antagonistic to the growth of the organisms. The medium was adjusted so as to give a final reaction of 0.3 per cent acidity to phenolphthalein. For each of 5 patients who had typical attacks of dengue, six culture tubes, three of each kind, were inoculated with whole blood taken on the first, second (2 cases), third, and fifth (2 cases) day of the disease, the blood being cultured immediately after it was withdrawn. After inoculation, the tubes were covered with about 2 cc. of sterile paraffin oil. Some of the tubes were incubated at 37°C., others at room temperature. At the end of five days, and at intervals of two or three days thereafter, the culture material was very thoroughly examined under a dark field microscope, but although the majority of the tubes remained uncontaminated for several weeks, until discarded, no evidence of the development of a Leptospira, or of any other organism which could be considered as having any relation to dengue, was discovered. Two guinea-pigs were inoculated intraperitoneally with 1 cc. of the culture medium from one case after five days and ten days incubation respectively, and another with the culture medium of a second case after five days incubation, but with uniformly negative results. Four culture tubes, two of each kind, were inoculated with blood from guinea-pig 3 (see above) but also without result. Other guinea-pigs were inoculated subcutaneously and by mouth with the culture material after five days incubation. All results were negative.

Conclusions concerning the Leptospira theory

In view of the consistently negative results which we have obtained in our search for a Leptospira-like organism by means of dark field examinations of fresh blood from patients and from inoculated animals, and by means of cultural methods which are successful with known species of Leptospira, we consider it improbable that the causative organism is of spirochetal nature. This conclusion is strengthened by a number of other considerations. Guinea-pigs are readily susceptible to the known kinds of Leptospira (*L. icterohemorrhagiae*, *L. icteroides*, *L. hebdomadis*), but are apparently quite immune to the virus of dengue. Moreover, in all the known Leptospira diseases (yellow fever, infectious jaundice, Japanese seven-day fever) the liver and kidneys are the organs most uniformly and injuriously attacked, resulting in jaundice and albuminuria, with excretion of the parasites with the urine, but in dengue neither the liver nor kidneys are ordinarily attacked in uncomplicated cases. The fact that Cleland Bradley and MacDonald were able to produce the disease by inoculation of blood which had been kept in an ice chest for as long as seven days also argues against the organism being a Leptospira since the latter is extremely short-lived after the death of the host, especially so in the case of *Leptospira icteroides* of yellow fever. The Leptospira theory is based solely on the supposed relationship between dengue and yellow fever, and as we have already shown, this relationship is not as close is commonly assumed. The fact that *Aëdes aegypti* is the principal if not the only transmitter of dengue as well as of yellow fever can hardly be construed as an important argument in favor of the close relationship of the organisms involved in the two diseases. As Noguchi has pointed out, *Aëdes aegypti* probably owes its distinction as the sole carrier of yellow fever to the fact that its gut offers the two conditions necessary for the growth of the causative organism; namely, sterility so far as bacteria are concerned, and the presence of at least minute quantities of blood or serum for food. That these same factors might favor the growth of other organisms, not necessarily Leptospirae, is by no means improbable. It is of interest to note that administration of arsphenamin did not have any effect in warding off an attack of dengue. Numerous cases developed in patients who were under intensive treatment for syphilis.

III. TRANSMISSION

Historical résumé

Since Graham (1903) first demonstrated the transmission of dengue by the bites of mosquitoes, there has been abundant confirmation of the fact that the disease is transmitted principally, and probably exclusively, by these insects. There is, however, some discrepancy in the results which have been obtained by different investigators as regards the species of mosquitoes involved in the transmission. Until 1916 it was generally accepted that *Culex quinquefasciatus* (= *C. fatigans*) was the principal if not the sole transmitter. This was based very largely on the work of Graham (1903) and Ashburn and Craig (1907). Graham, working at Beirut in 1901, infected 3 out of 4 men in unattacked houses within the epidemic area by exposing them to the bites of mosquitoes taken from the bed net of a dengue patient; the uninfected individual had had an attack of dengue two years before, as had Dr. Graham, and both were apparently immune. Graham also succeeded in infecting 2 individuals in a mountain village where no other cases occurred either before or after, by means of infected mosquitoes transported from Beirut. In all of these experiments Graham states that the majority of the mosquitoes utilized were Culex, yet in almost all, if not every case, "Stegomyiae" were among them. In 1902, while working on the life cycle of his supposed parasite in the mosquitoes, Graham states that the work was done almost wholly with "*Culex fatigans*" because it was easier to use in experimental work. In the course of this work 2 infections are recorded. In one case, Mrs. Graham was accidentally bitten by a mosquito infected fifteen days previously and developed a case of dengue after three days' incubation. In the other case, a man was subcutaneously inoculated with the salivary glands of a mosquito infected twenty-seven days before and developed a very severe attack after three days' incubation, but as Cleland, Bradley and MacDonald (1916) have pointed out, Graham seems to have been in some doubt as to the attack having been dengue, and bases his diagnosis largely on the finding of his supposed parasites in the blood. Graham does not definitely state that the mosquito which infected Mrs. Graham was a Culex. His meager remarks on the distribution of *Culex quinquefasciatus* and *Aëdes aegypti* in Beirut and its environs seems to indicate a closer correspondence with the distribution of dengue in the case of the latter species than of the former.

Ashburn and Craig (1907), working in the Philippines, made 9 mosquito transmission experiments using *Culex quinquefasciatus*, and had one successful inoculation. Three of the men used were subsequently shown by blood inoculation to be immune; another had probably previously had an attack, which, we think, would not necessarily have conferred immunity, while 3 others were apparently partially immune, since they developed mild cases, or had a prolonged incubation period, when subsequently inoculated with dengue blood. The successful case, who was not previously exposed so far as known, was placed under a mosquito net on September 12 with Culex mosquitoes which had bitten a patient on the night of September 11, but was not bitten until the night of September 13. This patient developed dengue symptoms on September 17 but had fever twenty-four hours earlier. It is significant, also, as has been pointed out by others, that this patient registered a temperature over 99°F. several times between September 13 and September 16. Although this single case is suggestive, it does not appear absolutely conclusive, since the experiment was performed in an epidemic area, and the possibility of his having been bitten before September 12, by infected *Aëdes aegypti* cannot, so far as we can gather from Ashburn and Craig's report, be absolutely excluded.

On the other hand, Cleland, Bradley and MacDonald (1918, 1919) were successful in transmitting the disease by means of *Aëdes aegypti* to 4 out of 7 volunteers, but failed in 3 instances in which *Culex quinquefasciatus* was used; their experiments were carried out in a non-epidemic area, and are therefore not subject to the suspicion which always rests to some extent on experiments carried out in the midst of an epidemic. The Australian work is further confirmed by Bancroft's (1905) transmission experiments, though these, by themselves, are inconclusive, since this work, like Ashburn and Craig's, was done in an epidemic area. The evidence against *Aëdes aegypti* furnished by transmission experiments is strikingly corroborated by epidemiological evidence furnished by Legendre (1911) in Indo China, by Lalor (1913) in Rangoon, by Archibald (1917) in Sudan, and by Stefko (1917) in Turkey.

Koizumi, Yamaguchi and Tonomura (1917), according to a review of their work by R. G. Mills, performed a large number of mosquito transmission experiments within an epidemic area in Formosa, using volunteers who were not under observation or confinement before the experiments were begun. They obtained positive results with *Aëdes* (*Stegomyia*) *scutellaris* and *Armigeres* (*Desvoidya*) *obturbans*, and consider the former species the principal transmitter; positive results were never obtained with *Culex quinquefasciatus* (*fatigans*). No mention of *Aëdes aegypti* is made. *Aëdes* (*Stegomyia*) *scutellaris* is very closely related to *Aëdes aegypti*, and has been suggested as a possible vector for yellow fever in the Orient, if yellow fever should be introduced into its area. *Armigeres* (*Desvoidya*) *obturbans* also belongs to the Aëdes group of mosquitoes. The incrimination of these mosquitoes instead of *Culex quinquefasciatus* in Formosa, where *Aëdes aegypti* is of such sporadic occurrence as to be a negligible factor in disease transmission, is highly suggestive.

Epidemiological evidence in the Texas epidemic of 1922

As was remarked at the beginning of this paper, the recent epidemic in Texas and neighboring states was accompanied by an unprecedented scourge of yellow fever mosquitoes (*Aëdes aegypti*). In Galveston and Houston, where the dengue epidemic first appeared and where it reached such astonishing proportions, the number of yellow fever mosquitoes was so unusually excessive as to have constituted a real plague. Numerous old inhabitants who knew nothing about species of mosquitoes or their relation to disease stated in reply to inquiries that they had been besieged by a new kind of mosquito with striped legs which bit in the daytime, and which they had never observed before. *Aëdes aegypti* is, as a matter of fact, a constant inhabitant of the gulf coast cities, but in ordinary years is far less abundant than *Culex quinquefasciatus,* and ordinarily escapes notice. The fact that the presence of a "new kind of mosquito" had forced itself upon the notice of individuals who were not accustomed to making observations of this kind is in itself highly significant. The scourge of these mosquitoes spread inland, and they appeared in enormous numbers in many places where they had seldom or never been observed before. They became abundant in such places as Fort Worth, Dallas, Waco, Austin, and San Antonio. Most estimates indicate that *Aëdes aegypti* constituted about 50 per cent of the mosquito population of these cities, in all cases an excessive average. Sometimes none are seen during an entire summer. In these cities, as in Houston and Galveston, they were frequently noted by old inhabitants as an entirely new thing. In ordinary years *Culex quinquefasciatus* is by far the most abundant house mosquito in the cities and towns throughout Texas, and appears to be the most generally distributed species. In Louisiana, where *Aëdes aegypti* is ordinarily more abundant than in Texas, there appears to have been a similar overproduction of these mosquitoes. Scott (1923) in his report of the dengue outbreak in that state says, "During the survey, Aëdes, gorged with the blood of dengue patients or in the process of feeding, was observed very often."

Numerous inquiries have been made to find out whether there was any town in the State in which dengue occurred where the absence of *Aëdes aegypti* had been noted by competent observers, but no such place could be located. The following report by Mr. E. W. Steel, Sanitary engineer for the International Health Board, engaged in anti-malarial work, relative to the prevalence of *Aëdes aegypti* and *Culex quinquefasciatus* and incidence of dengue in towns in East and Central Texas, where he was able to make personal observations, is of interest in this connection.

Navasota. Aëdes present in appreciable numbers throughout the summer. For several weeks in September it was undoubtedly the predominant species. Thereafter, Culex assumed the lead. Probably one-third of the inhabitants of Navasota had dengue.

Hearne. Aëdes and Culex both present, probably in equal numbers. Incidence of dengue about 30 per cent.

Rockdale. Aëdes present; Culex predominant. Dengue incidence about 40 per cent.

Conroe. Culex predominant. Aëdes present but not in great numbers. Dengue incidence about 25 per cent.

Gladstell. No Aëdes. Very few Culex. *Psorophora ciliata* occasionally seen. No dengue except 2 cases whose history showed that they were contracted elsewhere. In the town of Cleveland, three miles away, practically every one had dengue.

Livingston. Aëdes present. Dengue incidence about 40 per cent.

Trinity. Aëdes present in small numbers. Six cases of dengue occurred in the family of the hotel keeper and among permanent boarders at the hotel. Was never able to find any Aëdes in or around this hotel. Culex numerous. Dengue incidence throughout the town 30 per cent. Reports by the local mosquito inspectors show that dengue was more prevalent in the negro section where water barrels were most abundant.

Groveton. Aëdes present; Culex predominant; *Psorophora ciliata* also collected at edge of town. Dengue present in Groveton but have no figures as to prevalence.

Terrell. Aëdes and Culex present in great numbers with Aëdes predominating. Dengue incidence about 90 per cent.

Denton. Aëdes present. Dengue incidence about one-third.

Austin. Aëdes present. Dengue incidence about 20 per cent.

During the last half of June and during July, *Aëdes aegypti* was found 90 times in rooms occupied by dengue patients in Galveston, in many instances by hundreds. *Culex quinquefasciatus* was found together with Aëdes 5 times, and alone 1 time. During August Culex became more common, and in September the two were found in almost equal numbers. In Houston, during the month of October, the rooms of 12 dengue patients were examined in various parts of the city and Aëdes found in all but one. In this case no mosquitoes could be found, nor had any been observed by the family. The patient, however, had spent a number of days, but not nights, with a daughter at one of the Houston hospitals during the preceding week. Aëdes, but no Culex, were found in every room and in every hallway in this hospital in which a search was made, and every one of the nurses had been attacked by the disease, as well as many of the patients. In two hospitals at Galveston in which severe epidemics occurred, yellow fever mosquitoes were seen every morning during the summer, whereas only two or three Culex were seen, on seven occasions.

At the Houston Tuberculosis Hospital not a single case of dengue was contracted. This hospital is situated apart from other buildings in an open piece of country. Culex was present and there were a few Anopheles, but no Aëdes were seen.

In the Rice Institute Residential Halls, which are also situated in a more or less isolated place, Culex was present in unusually large numbers, and constituted more of a scourge than in any other part of the city, so far as we can determine. Three hundred and fifty students were in residence in the three halls, and complained bitterly of the plague of mosquitoes, which swarmed by hundreds in the halls and rooms, making it necessary to sleep under bed nets. From two to four students occupy single rooms or suites, so that the conditions, if *Culex quinquefasciatus* were an efficient transmitter of dengue, were ideal for wholesale infection.

It is inconceivable that any student residing in the halls could have escaped being bitten by infected mosquitoes. *Aëdes aegypti* was also present, but in East Hall and South Hall, where frequent mosquito collections were made, there was not more than 1 Aëdes to from 25 to 50 Culex, and in many of the rooms no Aëdes at all could be found. It is probable that the few which were present were not bred on the premises, since a careful search for Aëdes larvae was fruitless; more likely they were carried out from town in automobiles and in clothing. Unfortunately, no mosquito collections were made in West Hall, which shows a markedly higher percentage of dengue cases. The incidence of dengue in these three dormitories was as follows:

STUDENTS IN RESIDENCE		DENGUE CASES	PERCENTAGE
			per cent
99	East Hall:		
	First floor	2	
	Third floor	5	
	Total	7	7.1
126	South Hall:		
	First floor	4	
	Second floor	4	
	Third floor	3	
	Fourth floor	2	
	Total	13	10
125	West Hall:		
	First floor	1	
	Second floor	6	
	Third floor	16	
	Fourth floor	2	
	Total	25	19.8

As compared with the dengue incidence in Houston as a whole (estimated at 20 per cent), these figures, even those from West Hall, are significant, in consideration of the unexcelled opportunities for the spread of the disease if Culex were a transmitter. The slight extent to which the disease spread within the halls is emphasized by the fact that a considerable number of the cases were undoubtedly contracted in town, and by the very significant fact that in not a single instance in East Hall and South Hall did two cases occur in the same room, while in West Hall there were only three instances of two infections in one room, all on the third floor.

In Houston it is probable that the stock supply of Aëdes originated at the wharves on the Ship Channel. Here there were found several hundred fire barrels teeming with larvae of both Aëdes and Culex, but at least 10 of the former to 1 of the latter. The men working in the offices on the docks were forced to move their desks out into the open where a strong breeze swept through, finding it impossible to work inside. On a single coat hanging in a dark corner of one of these offices not less than 200 yellow fever mosquitoes were seen, and under the tables and desks there was practically no mosquito standing room left. A Culex was only rarely seen. It is probable that the countless hordes of hungry mosquitoes bred in this place were carried all over the city in automobiles, freight cars, etc., and furnished the initial supply which bred wherever suitable breeding places could be found. It is hardly necessary to add that practically every employee at the wharves was attacked by dengue.

Medical students who returned to Galveston on the first of October, at which time the epidemic was diminishing, concomitant with a 50 per cent decrease in numbers of Aëdes and a steadily increasing supply of Culex, showed a dengue incidence of 7 per cent, whereas the city had an incidence of about 35 per cent.

From the epidemiological evidence set forth above, we are forced to the conclusion that, so far as the Texas epidemic is concerned, *Aëdes aegypti* was the primary factor in the transmission of the disease. While we are not prepared to say that *Culex quinquefasciatus* also may not, under some circumstances, be capable of transmission, we think that the evidence is sufficient to show that this mosquito at most plays a minor part, and can not be as efficient or frequent a transmitter as is the yellow fever mosquito. We consider it highly improbable that the epidemic would ever have developed in the absence of *Aëdes aegypti* in considerable numbers. Dengue is known as a coastal disease, and a severe epidemic has never before affected the inland parts of Texas. The occurrence of such an epidemic as that of 1922 concomitant with an unprecedented scourge of *Aëdes aegypti* appears to us to be more than coincidence.

Transmission experiments

In order to show conclusively that *Aëdes aegypti* was really the transmitter of dengue in this epidemic, we planned a number of transmission experiments, such as our resources and opportunities would permit. We had also hoped to carry on experiments to show the relation, if any, of *Culex quinquefasciatus* to the disease and the duration of infectiveness in the mosquito and the human host, as suggested to one of us by Colonel Craig. Most of these experiments could not successfully be carried out, our work being frustrated by a number of unforeseen and unavoidable misfortunes and difficulties. We sent infected mosquitoes to a number of communities where dengue had not occurred, for use in transmission experiments, but either the mosquitoes died en route, were destroyed by ants, or could not be induced to bite on arrival. In two communities, dengue appeared for the first time that it had ever been known to occur there, before the arrival of the infected mosquitoes. Further efforts along this line were frustrated by the dying out of the epidemic, after arrangements had been made for these experiments.

One of us (L. R.) succeeded in carrying out a few experiments with volunteers in Galveston. After eight volunteers had been found with great difficulty, no other suitable person could be found for two weeks. A personal attack of the disease then supervened after which the entire system of isolation and nursing had disappeared, funds, which had been personally supplied, were exhausted, and these experiments had to be abandoned.

For the Galveston experiments volunteers were found among new arrivals on evening trains, and were immediately placed in carefully screened rooms, where they were observed for ten days, during which time blood counts, urine analyses, and Wassermann tests were made. Except for minor non-infectious injuries, all of the volunteers were found to be in good physical condition. Two women had come direct from Colorado Springs, Col., two men from Denver, Colo., one man from Albuquerque, New Mexico, two from Yellowstone Park, and one from Leadville, Colo., the statements of the volunteers being corroborated by baggage checks or pullman slips. During the period of observation the temperature and pulse were taken twice daily, and as soon as prodromal symptoms of dengue appeared they were taken three times daily. No drugs of any kind were given, but ice bags were used for severe headaches, and the patients were frequently allowed to take 10 grains of milk sugar in a capsule for pain. Strange to say, this simple powder seemed to afford as much, or as little, relief as the salicylates and phenacetin given to other patients. The experiments were performed at a private house where good and intelligent coöperation was received. The copper screens were doubled with mosquito bar, and every one watched for mosquitoes indoors. A total of three were seen and killed during the course of the experiments. None of the volunteers left the second floor, but they visited each other on that floor, and had their meals served in their rooms. The laboratory-bred mosquitoes used in the experiments were released under a closely gathered net with the volunteer stripped to the waist, 30 to 40 mosquitoes being used in each instance. The mosquitoes were seen to bite in every case recorded as a bite, the sting was noted by the volunteer, and a papule was observed; we cannot, of course, be certain that in some instances the same mosquito did not bite twice. After a number of bites had been recorded, all the mosquitoes were killed.

Details of experiments

Case 1. Alexander Knox, male, aged twenty-three years, mechanic, arrived 9:30 p.m. July 24, 1922, from Albuquerque, New Mexico, and was taken to a screened room where he remained constantly. Physically well, excepting a second degree burn of right hand. Wassermann negative. Urine negative. Blood count, 5,200,000; Hb.—102, W.B.C.—7900; P—64, L—28, T—3, L.M.—3, E—2.

On August 3, 1922, at 2:30 p.m. he was bitten by 17 Aëdes which had fed, twenty-four hours before, on a dengue patient in the second day of the disease.

Temperature and pulse normal until August 9, 1922. Prodromal symptoms were present when he awoke on the morning of the ninth, although he was well when he retired at 10 p.m. the night before, at which time five days and seven and one-half hours had elapsed. The actual onset with fever occurred during the afternoon of the ninth, the temperature being 99.5°F. at 2:00 p.m., at which time approximately six days had elapsed.

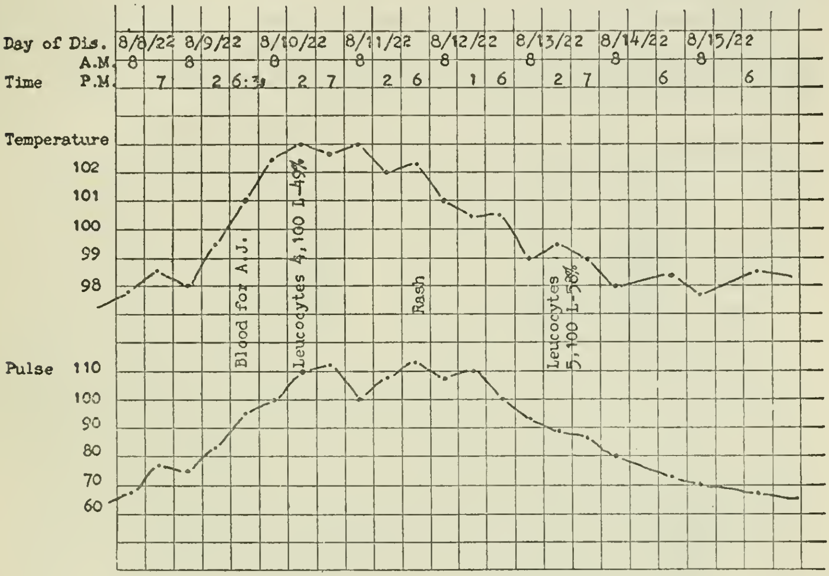


CHART NO. 1 Alexander Knox.

At 6:30 p.m. August 9, 1922, 1 cc. of blood was taken from an arm vein and immediately given intravenously to Annie Jackson.

August 10, 1922, leucocytes 4100, lymphocytes, 49 per cent.

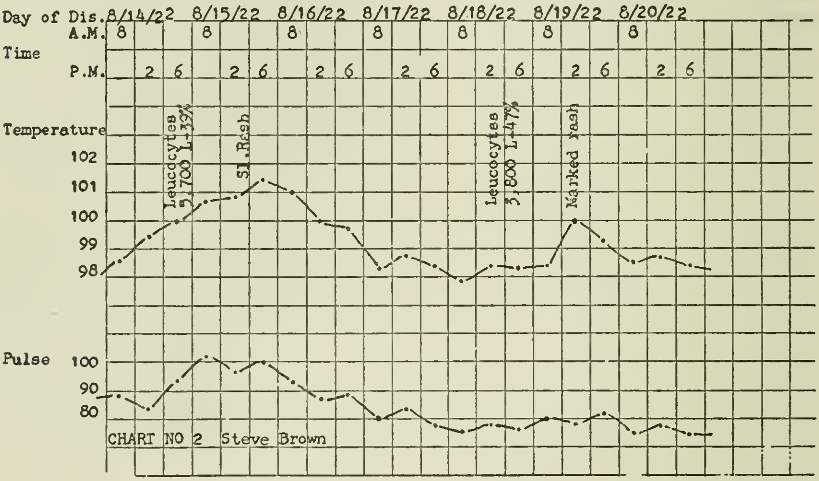
August 13, 1922, leucocytes 5100, lymphocytes, 58 per cent.

The patient ran a typical course, a definite rash appeared on the 11th, and he left his quarters on August 15.

Case 2. Steve Brown, male, aged thirty years, laborer, arrived 9:30 p.m., July 29, 1922, from the Yellowstone Park, and was taken to a screened room where he remained. Physically well, excepting a small hernia. Wassermann negative. Urine negative. Blood count 4,940,000; Hb.—100, W.B.C.—8300; P—64, L—32, T—2, L.M.—2.

On August 8, 1922, at 8:00 a.m., he was bitten by 5 Aëdes which had fed, forty-eight hours before, on a dengue patient, in the third day of the disease.

Temperature and pulse normal until August 14, 1922. Prodromal symptoms appeared at noon, and the temperature at 2:00 p.m. was 99.4°F. Six days and four hours had elapsed at noon.



August 14, 1922, leucocytes 5700, lymphocytes, 39 per cent.

August 18, 1922, leucocytes 3800, lymphocytes, 47 per cent.

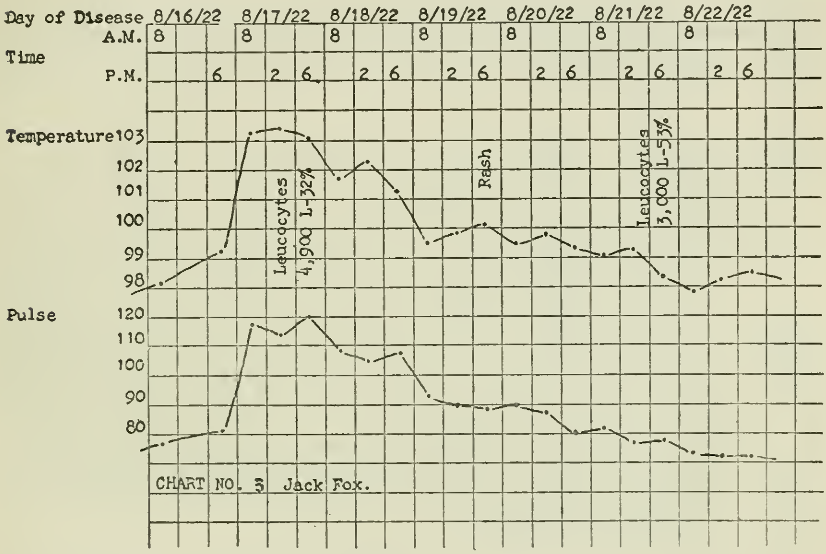
The patient had a rather light attack of dengue with a slight rash on August 15, and a well-marked rash on August 19 with a relapse of short duration.

On August 15, 1 cc. of blood was withdrawn from an arm vein and immediately given intravenously to Jack Cooge (No. 8).

Case 3. Jack Fox, male, aged twenty seven years, mechanic, arrived 8:40 p.m. July 31, 1922, from Denver, Colo., and was taken to a screened room where he remained. Physically well excepting a severe wrench of the left knee. Wassermann negative. Urine negative. Blood count 4,870,000; Hb.—92 per cent; W.B.C.—6700; P—69, L—30, E—1.

On August 10, 1922, at 9:00 a.m. he was bitten by 19 Aëdes which had fed, seventy-two hours before, on a dengue patient in the fourth day of the disease.

Temperature and pulse normal until August 16. At 6:00 pm., August 16, 1922, his temperature was 99.2°F, but he felt perfectly well, and probably would not have noticed a slight headache and stiffness if I had not told him to watch for and note the time of any symptoms.



He was the only experimental case that had a temperature preceding symptoms. Prodromal symptoms were noticed at 9:00 p.m. August 16, 1922. Six days and twelve hours had elapsed.

August 17, 1922, leucocytes 4900, lymphocytes, 32 per cent. Symptoms well marked.

August 21, 1922, leucocytes 3800, lymphocytes, 53 per cent.

A rash which was present on the morning of August 19 had spread over the entire body by mid-afternoon, and the patient was very miserable. The attack varied in no way from that usually seen in dengue, and was moderately severe.

Case 4. May Rounds, female, aged thirty-one years, saleslady. Arrived August 8, 1922, 9:00 p.m. from Colorado Springs., Colo., and was taken to a screened room where she remained. Physically well excepting a painful sprain of the left ankle. Wassermann negative. Urine negative. Blood count 4,560,000; Hb.—98 per cent; W.B.C.—8700; P—61, L—36, T—2, E—1.

On August 19, 1922, at 4:00 p.m. she was bitten by 10 Aëdes that had fed, ninety-six hours before, on a dengue patient in the fifth day of the disease.

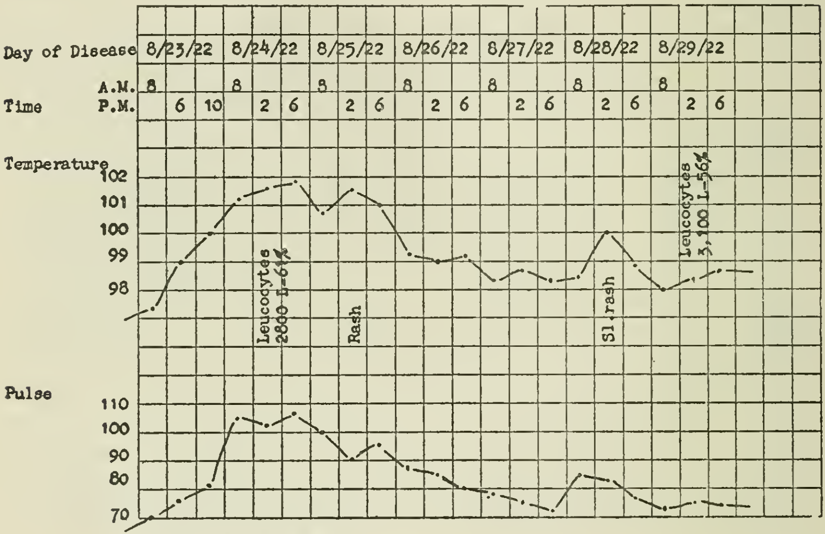


Chart No.4 May Rounds.

Prodromal symptoms appeared August 23, 1922, at 6:00 p.m. at which time the temperature was 99°F., and at 10:00 p.m. the temperature was 100°F. Four days and two hours had elapsed. This was the shortest incubation period in our cases.

August 24, 1922, leucocytes 2800, lymphocytes, 61 per cent.

August 25, 1922, rash on abdomen, back, and flexor surfaces. Blood taken for Bailey Sanders.

August 28, 1922, relapse and secondary rash.

August 29, 1922, leucocytes 3100, lymphocytes 56 per cent.

This case presented the so-called text-book picture with a slow pulse and a saddle-back temperature curve.

Case 5. Bailey Sanders, male, aged twenty-six years, switchman, arrived 9:30 p.m. from Leadville, Colo., and was taken to a screened room. Physically well. Wassermann negative. Urine negative. Blood count 4,900,000; Hb.—102 per cent; W.B.C.—9100; P—63, L—35, E—2.

On August 11, 1922, 2:00 p.m. was bitten by 9 Aëdes that had fed, ninety-six hours before, on dengue patient in fifth day of the disease.

He remained well, and although a similar experiment had been repeated on May Rounds the day before it was feared that a volunteer was being wasted, so on August 20, 1922 nine days later, 3 Aëdes bit him which had fed the day before on an active case in the second day.

He remained well, and on August 25, 1922, he received 1 cc. whole blood intravenously from May Rounds. He remained well and has not had dengue.

Case 6. J. S. F., male, aged thirty-seven years, carpenter, arrived from Denver, Colo., 9:00 p.m. August 3, 1922, and was taken to a screened room. Physically well excepting a small cut on right hand. Wassermann and urine negative. Blood count 5,400,000; Hb.—100 per cent; W.B.C.—8300; P—68, L—26, L.M.—4, E—2.

August 13, 1922, at 2:00 p.m. bitten by 4 Aëdes and at 4:00 p.m. by 7 Aëdes, which had fed ninety-six hours before on a dengue patient in the fifth day of the disease. This was a repetition of no. 5, and was subsequently repeated in no. 4 with success.

August 19, 1922, he deserted his contract and went to work. He was feeling well, and he would not remain indoors. I saw him daily for several days, and he showed neither symptoms nor temperature, but on September 15, 1922, he passed through a mild attack of dengue and returned to work on the fifth day.

Intravenous inoculation with whole blood

Case 7. Annie Jackson, female, aged nineteen years, seamstress, arrived 9:00 p.m. July 30, 1922, from Colorado Springs, Colo. Wassermann was negative, but I was in some doubt, and she was held for injection experiments. Urine negative. Blood count 4,620,000; Hb.—88 per cent; W.B.C.—7900; P—58, L—35, L.M.—2, T—3, E—2.

August 9, 1922, 6:30 p.m. given intravenously 1 cc. whole blood withdrawn a moment before from Alexander Knox who had the initial symptoms of dengue fever induced by bites of Aëdes which had fed on a dengue patient.

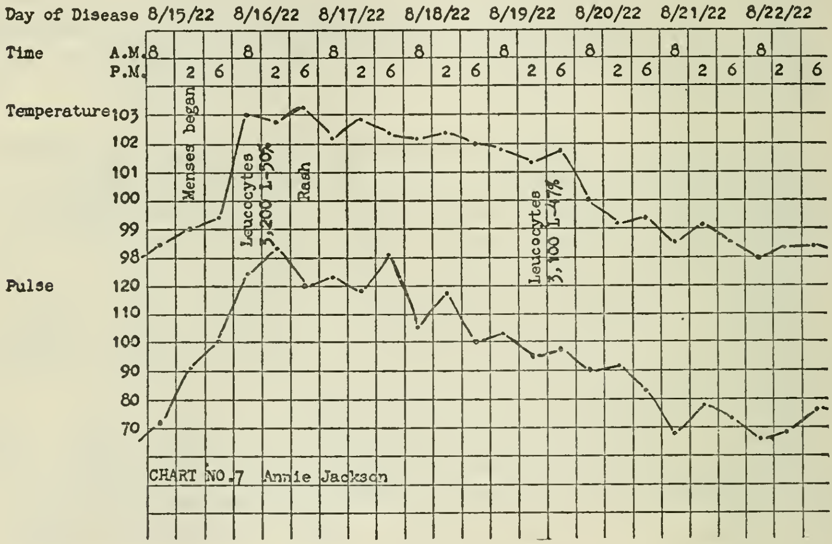
Felt well. Temperature and pulse normal until August 15, 1922.

August 15, 1922 prodromal symptoms appeared at 1:30 p.m. Temperature at 2:00 p.m. 99°F., pulse 91; five days and nineteen hours had elapsed. Menses began, ten days too soon.

August 16, 1922, leucocytes 3200, lymphocytes 50 per cent.

August 19, 1922, leucocytes 3100, lymphocytes 47 per cent.

This patient had a rather severe illness which ran the course of dengue fever. She had a prolonged convalescence. The menstrual flow was excessive, and she was very weak for ten days. Discharged September 5, 1922.



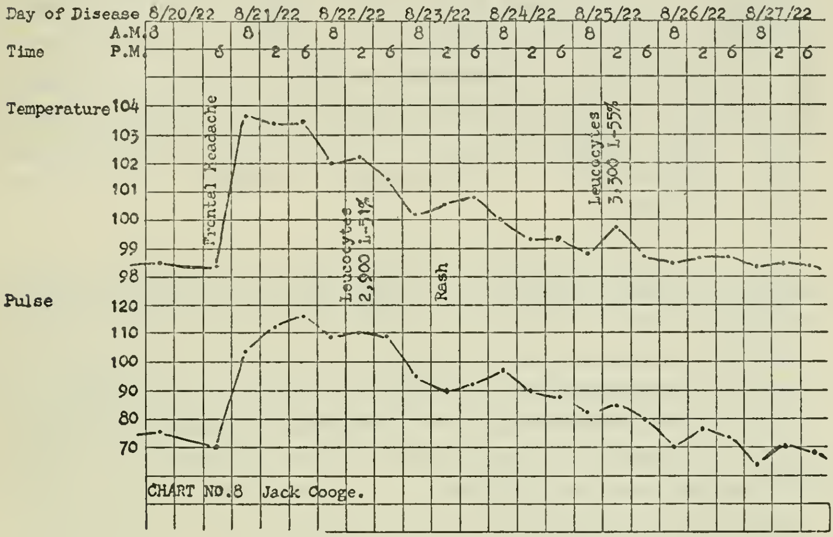
Case 8. Jack Cooge, male, aged twenty years, mechanic, arrived 9:30 p.m. July 29, 1922, from the Yellowstone Park and was taken to a screened room where he remained. Physically well, but he gave a history of chancre two years ago followed by vigorous treatment. Scar present. Wassermann negative. Held for intravenous inoculation. Urine negative. Blood count 5,300,000; Hb.—101 per cent; W.B.C.—6900; P—67, L—27, L.M.—3, E—3.

August 15, 1922, 2:00 p.m. given intravenously 1 cc. whole blood which had been withdrawn, a moment before, from volunteer Steve Brown who had noticed the first symptoms of an experimental case of dengue fever about twenty-six hours before.

August 20, 1922, 8:00 a.m. Temperature and pulse normal.

August 20, 1922, 6:00 p.m. Temperature 98.4°F., pulse 70, but he was nauseated, did not eat supper, and had a severe frontal headache which he noticed at 5:00 p.m. Five days and three hours had elapsed.

August 21, 1922, at 8 a.m., temperature 103.6°F., pulse 104, face and neck flushed, aching pains; this patient had a subsequent course which was typical of dengue fever.



Summary of experiments

Dengue fever was successfully transmitted to 4 out of 6 volunteers by *Aëdes aegypti*, as follows:

Case 1. Twenty-four hours after biting patient in second day of disease. Incubation period approximately five days and eighteen hours.

Case 2. Forty-eight hours after biting patient in third day of disease. Incubation period six days and four hours.

Case 3. Seventy-two hours after biting patient in fourth day of disease. Incubation period six days and twelve hours.

Case 4. Ninety-six hours after biting patient in fifth day of disease. Incubation period four days and two hours.

Of the 2 volunteers to whom the disease was not transmitted by infected mosquitoes, one (no. 5) was bitten by mosquitoes fed ninety-six hours previously on a patient in the fifth day of the disease and again nine days later by mosquitoes fed twenty-four hours previously on a patient in the second day of the disease. Finally, five days later, he was intravenously inoculated with 1 cc. of blood from no. 4 taken about forty-four hours after onset of the disease, but was not infected and did not subsequently contract the disease. The other unsuccessful case (no. 6) was bitten by mosquitoes fed ninety-six hours previously on a patient in the fifth day of the disease. He had a mild attack of the disease twenty-seven days after being released.

The experiments with intravenous inoculation of blood resulted as follows:

Case 7. Inoculated with 1 cc. blood from no. 1 four and one-half hours after onset; positive. Incubation period five days and nineteen hours.

Case 8. Inoculated with 1 cc. blood from no. 2 about twenty-four hours after onset; positive. Incubation period five days, 3 hours.

Case 6. Inoculated with 1 cc. blood from no. 4 about forty-four hours after onset. Previously bitten by two batches of mosquitoes with negative results. Negative.

While these experiments were carried on in an epidemic area, and are therefore not so absolutely conclusive as they would have been if they could have been carried on in an uninfected place, we feel reasonably certain that the results can be relied upon in view of the precautions which were taken to prevent outside infection. We conclude, therefore, that the disease, as it appeared in the Texas epidemic, was transmissible by *Aëdes aegypti* in from twenty-four to at least ninety-six hours after biting a patient, and that these mosquitoes can become infected from patients in various stages of the disease from the first to at least the fifth days. Our blood inoculation experiments show that the blood is infective by direct inoculation four and one-half and twenty-four hours after onset. The incubation period, up to the time of appearance of prodromal symptoms, varied from four days and two hours to six days and twelve hours, and was not shorter in patients infected by inoculation of 1 cc. of blood than in patients infected by mosquitoes. This conforms fairly well with the findings of Koizumi, Yamaguchi and Tonomura, who state that the average incubation period is 130 hours; with Ashburn and Craig's single case, which had an incubation period of about four days; and with Graham's cases, which developed in from three to six days. Cleland, Bradley and MacDonald's experimental cases in Australia developed in from five one-half to nine one-half days, in the majority of cases being about eight days, which seems to indicate that they were working with a slightly different strain of the disease.

IV. SUMMARY

1. An epidemic of dengue of unprecedented proportions originating in Galveston in June, 1922, spread over all of eastern and central Texas, and into neighboring states. Between 500,000 and 600,000 cases were estimated to have occurred in Texas.

2. The organism of dengue, shown by previous workers to be a filtrable virus in the blood plasma, has been suspected of being a Leptospira on account of a supposed relationship of the disease to yellow fever. This relationship we believe to have been overemphasized.

3. Dark field examinations of blood smears, inoculations into guinea-pigs, mice and monkeys, and attempts to cultivate the organism in media used for Leptospirae, have all given negative results.

4. Negative findings in blood, in inoculated animals (particularly guinea-pigs), and in cultures; the usual absence of liver and kidney involvements; the fact that the relationship of dengue to yellow fever has been overemphasized; and the viability of the virus in blood kept in an ice chest after removal from the host (according to Cleland, Bradley and MacDonald) lead us to believe it improbable that the organism of dengue is a Leptospira.

5. Previous experimental work and epidemiological evidence point to *Aëdes aegypti* (*-Stegomyia fasciata*) as the principal

transmitter of dengue, this species being replaced principally by *Aëdes* (*Stegomyia*) *scutellaris* in Formosa. *Culex quinquefasciatus* (*fatigans*) has been incriminated on very slender evidence. The Texas epidemic was accompanied by an unprecedented scourge of *Aëdes aegypti,* and the epidemiological evidence points very strongly to the agency of this mosquito in transmission, and suggests that *Culex quinquefasciatus* played a very minor part, if involved at all.

6. Transmission experiments carried out with *Aëdes aegypti* were successful in 4 out of 6 cases; the mosquitoes succeeded in transmitting the disease in from twenty-four to ninety-six hours after feeding on patients in the second to fifth days of the disease.

7. The incubation period in experimentally infected cases, both when bitten by infected mosquitoes and when inoculated with a patient's blood, varied from four days and two hours to six days and twelve hours.

SUMARIO

1. En junio del 1922 se presentó en Galveston una epidemia de dengue de proporciones sin precedente la que se extendió por toda la región oriental y central de Tejas y a los Estados vecinos. Se calcula que solamente en Tejas ocurrieron de quinientos a seiscientos mil casos.

2. Varios investigadores al demostrar que el organismo del dengue es un virus filtrable del plasma sanguíneo, han sospechado que aquel sea un Leptospira debido a la supuesta relación de esta enfermedad con la fiebre amarilla. Nosotros creemos que a esa relación se le ha dado demasiado importancia.

3. Los exámenes de sangre al ultramicroscopio, las inoculaciones de conejillos de indias, ratones y monos, y todos los esfuerzos por obtener dichos organismos en los medios de cultivo propios para los Leptospiras, han dado resultados negativos.

4. Los resultados negativos de la sangre, las inoculaciones, y los cultivos; la falta de afecciones hepáticas y renales; la creencia exagerada acerca de la relación que se supone exista entre el dengue y la fiebre amarilla, y la viabilidad del virus cuando se conserva en una atmosfera helada (esto último según las indica-

ciones de Clelland, Bradley y MacDonald) nos hacen creer que es improbable que el organismo del dengue sea un Leptospira.

5. El trabajo experimental previo y la evidencia epidemiológica indican al *Aëdes aegypti* (*Stegomyia fasciata*) como el principal agente transmisor del dengue, aunque en Formosa esta especie puede ser sustituída por el *Aëdes* (*Stegomyia*) *scutellaris*. La evidencia a favor del *Culex quinquefasciatus* es muy débil. Una plaga de *Aëdes aegypti*, de inmensas proporciones, acompañó a la epidemia de Tejas lo que nos hace creer que este mosquito es en realidad el agente transmisor al mismo tiempo que afirma la poca importancia del *Culex quinquefasciatus*.

6. Los experimentos de transmisión llevados a cabo con *Aëdes aegypti* tuvieron éxito.

7. De seis casos experimentales en los que se empleó el *Aëdes aegypti* como agente transmisor se obtuvo resultados positivos en cuatro. Los mosquitos causaron la enfermedad en períodos que variaban de las 24 a las 96 horas previa alimentación de aquellos sobre pacientes que ya tenían desde el segundo hasta el quinto dia de la enfermedad.

8. En todos los casos, ya infectados directamente permitiendo que el mosquito picara al paciente o mediante inoculacion de sangre infectada, se obtuvo un período de incubación variable entre los 4 días y 7 horas hasta 6 días y 12 horas.

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CORRESPONDENCE

March 16, 1923

Editor of the American Journal of Tropical Medicine:
Dear Sir,

I have transmitted to you under separate cover an article by Dr. Sh. Moshkovsky on the Tropical Institute in Moscow. This article (written in German) was presented to me by the author last August in Moscow, where I was making a visit for the purpose of investigating the condition of the biological sciences and scientists, and of helping to reestablish communication with them. An account of my findings will be published in the forthcoming number of the *Scientific Monthly.*

The Institute of Tropical Medicine, which I visited, comprises a large staff of energetic workers. They are actively engaged in research at the present time and are hampered in their work chiefly by their isolation. They have received practically no journals or books from America since 1917 and keenly feel the need of them. It is unavoidable under these circumstances that there will be much unnecessary duplication of work and wasted efforts at this Institute. The situation could easily be remedied if the workers in this line in America would send their reprints to the Moscow Tropical Institute and in addition all back numbers which they can spare of such journals as *Parasitology, Journal of Infectious Diseases, Journal of Tropical Medicine, Journal of Experimental Medicine,* etc. Besides this it would be extremely desirable to have the scientific societies in these fields send a subscription for the current issues of such journals of the Institute. This would not make a noticeable impression on their funds but would reach a large proportion of the Russian workers interested in these matters, owing to the peculiar centralization of Russian science at the present time. Mail may be sent directly now to the addresses in Russia. It is better to send the reprints and journals in care of the Institute rather than to a particular person as such, in order to be certain of avoiding import duty on the Russian side.

I might also mention here the Institute of Microbiology under Professor Barikin, which is doing valuable work on typhus, cholera and other diseases, including theoretical work in Immunology and in the effects of selection on microorganisms. This Institute is in sad need of foreign literature and communication. The address of both of them is 8 Kudrinskaya Place, Moscow. Anything that we could do to help reestablish inter-communication with these scientists would help the advancement of science in general. They too publish journals and various important monographs which we here should not be ignorant of, and they would doubtless desire in return for our articles to publish some of their work here.

Yours very truly,
H. J. Muller,
Associate Professor of Zoology,
The University of Texas.

SOCIETY NOTICES

ANNUAL MEETING

The American Society of Tropical Medicine will hold its regular annual meeting June 25 and 26, 1923 at San Francisco, California, where also during the same week the meeting of the American Medical Association is to be held. As the scientific sessions of the American Medical Association do not begin until Wednesday there will be no conflict in the programs of the two organizations, Monday and Tuesday being the meeting days of the Society of Tropical Medicine.

Members who desire to present papers should send titles at an early date to the Secretary so that the Program Committee can give them a suitable place on the program which it is expected will be printed and mailed to members on or about June 1.

B. H. Ransom, Sec.-Treas.

BOOK REVIEW

An Introduction to The Practice of Preventive Medicine. By J. G. Fitzgerald, M.D., F.R.S.C., Professor of Hygiene and Preventive Medicine and Director, Connaught Antitoxin Laboratories, University of Toronto.

This book comes from the press at a time when an ever increasing number of people are becoming interested in preventive medicine and public health work.

The volume contains 25 chapters covering 712 pages, in addition to which there are 90 pages of valuable appendices. The arrangement of the work as a whole is excellent; however, it is noted that leprosy is considered in the chapter on insect borne diseases, along with malaria, dengue, etc. On page 155, in the discussion of the emetine treatment of amebic dysentery, a statement is made concerning "vegetable" forms of ameba.

In the article dealing with hook-worm, thymol and betanaphthol are both mentioned as being used to clear up infestations of this parasite. No mention is made of oil of chenopodium or carbon tetrachloride.

The chapters on water, milk and foods are written by H. M. Lancester. A large amount of information is considered in a comparatively small space. In discussing botulism and the causative organism no mention is made of the various types and at the end of the article the statement appears "recent experiments on the development of a satisfactory antitoxin for purposes of treatment and of prophylaxis of botulism have not proved very successful." This statement, in so far as prophylaxis is concerned, is not borne out by experiment. It is true that where outbreaks of this disease have occurred antitoxin has not, in most instances, been available in time to prevent the onset of symptoms. In laboratory experiments, however, antitoxin does protect, and as the supply becomes available there is no reason why it should not be used with as great effectiveness as tetanus antitoxin.

The chapter on vital statistics is rather brief and at the end a table of the British "Standard Million" is given. There are no instructions as to the method of utilizing this table nor is the "Swedish Standard" mentioned.

There is a very valuable chapter on Public Health Organization and this chapter together with the appendices should be found of great value to health officers. Appendices contain extracts from various legislative measures relative to public health, specimen blank forms and detailed description of the various activities connected with the organization and administration of health centers.

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THE TREATMENT OF MALARIA

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In that admirable work, ''Endemic Diseases of the Southern States,'' which should be familiar to all who treat malaria, Deaderick has compiled a record of 5,109,001 authentic cases of malaria, showing 148,055 deaths, or a ratio of 2.89 per cent. Another table records 27,039 cases of pernicious malaria with 7,205 deaths, a ratio of 26.6 per cent. Upon reference to a number of American text books the mortality rate for the Southern States is found estimated at 3 per cent.

Malaria has such an extensive geographical distribution, with an almost epidemic concentration index in all warm latitudes, that in incidence it easily tops the list of diseases of man. As it attacks literally millions every year, the enormous toll of death which this Moloch of the tropics is constantly exacting may be readily seen. In fact, for a disease which has a specific treatment, the death rate is too high—something must be done about it.

Hundreds of earnest and competent students are giving their attention to the problem of malaria and the brilliant research work accomplished in this field is of common knowledge. Ask any young medical graduate about malaria and he will immediately recount in admirable detail the sanitary methods for its control, the differential characteristics of the plasmodia, and their individual life cycles in host and vector. Ask him concerning the treatment and he will invariably state simply quinine. Right about there his practical information usually stops, because quinine is the all-recognized specific. The latter is entirely true, yet the proper application of quinine is the essential thing. Even veteran practitioners in malarial districts often do not prescribe quinine according to the methods laid down by scientific research. Too frequently they give the patient a handful of quinine and leave him to take it, with the result that when his acute symptoms are gone he stops the medicine, his case passes to a chronic stage and he becomes a constant source of infection from which mosquitoes may transmit the disease to the community. The writer regrets that he personally knows physicians in the malarial areas of the South who never heard the gospel so beneficiently preached by Bass: "If all malaria cases could be treated until cured, by continuing the quinine in proper dosage for at least two months, malaria would cease to be a problem."

Sanitation has more than justified itself, but the overwhelming expense of instituting and maintaining sanitation amid the fierce vegetation of tropical lands, and the Herculean task of educating their lethargic native populations to the practice of communal cleanliness, removes the millennium of a sanitarized tropics too far distant for present contemplation. Panama and the few other similar spots are but a scratch upon the slate. Therefore, we can not escape the fact of the enormous malarial death rate which constantly stares us in the face, and the medical man is on trial.

We must turn our attention again to treatment, revise our knowledge of the measures at hand, and make serious application of these therapeutic principles in our practice. Malaria is not a disease to be taken lightly, and the haphazard exhibition of quinine is not sufficient. Every case should be individually studied, with as much interest as a case of tuberculosis would be, and an adequate treatment with proper isolation prescribed and supervised.

As medical officer for the past three years of a large industrial organization operating in the Lebrija river valley of Colombia, in the heart of the South American jungle, the writer has had the privilege of serving in a region dreaded throughout that republic because of its unusually severe malaria endemicity. During this time there has come under his personal observation over 8000 cases of malaria, in the proportions of quartan, 6 per cent; tertian, 44 per cent; and, estivo-autumnal, 50 per cent, as determined by microscopic blood examinations. The official hospital records of these cases show a mortality of 0.2 per cent, while the death rate for the pernicious types was 6 per cent.

In the belief that the system of treatment generally used would prove interesting to the profession, it is briefly outlined in this paper. The routine was adopted after studying the results obtained by a careful clinical trial of practically every method described in the literature. No claim for originality is intended. All theoretical and scientific explanations are avoided, it being desired to present in a practical and elementary way, a résumé of present malaria treatment for the convenience of the busy rural physician who battles heroically on the frontier, often without laboratories or libraries, and is, after all, the man in whose hands lies the conquest of malaria.

ACUTE MALARIA

Malaria is the diagnosis in 90 per cent of all sickness beginning with high fever in the tropical hot lands, except during the frequent mild influenza epidemics which invade the lowlands from the mountain districts where the latter disease is endemo-epidemic.

Paludism presents a wonderful variety of forms, and may at times closely simulate practically all other known diseases. For example there may be in malaria, pneumonia, meningitis, pleurisy, neuralgias, rheumatism, otitis media, coryza, stomach disorders resembling ulcer, appendicitis, diarrhea, typhoid, disturbances of vision, lumbago pseudo-angina pectoris, heart murmurs, hepatitis suggesting gall stones, severe pains in the spleen, pyelitis and kidney pains, cystitis or hematuria, generalized lymphadenitis, skin eruptions, extensive furunculosis, etc.

As a general rule in the malarial belt every patient, regardless of this presenting symptom, should have his temperature taken. Malaria patients in the beginning of the disease will often be unaware of the presence of fever, although the thermometer may show at that very minute a temperature of 104° or 105°F.—they do not feel it. The tongue should be scrutinized for the heavy, dirty-white, furred coating, an almost pathognomonic symptom, and the complexion examined for the peculiar faded-out color, very characteristic of paludism, and a careful history elicited concerning past malarial attacks.

If in doubt of the correct diagnosis, try the therapeutic test, viz., the treatment of malaria. It is surprising to see the great majority of cases clear up even after having resisted all other lines of treatment. One of our axioms is that in any case of obscure pain occurring in a patient in the malarial district, quinine in full doses is to be made the basis of treatment.

Treatment. 1. As soon as a diagnosis is made, give calomel 5 grains (0.32 gm.) together with quinine 15 grains (1.0). No other purgative seems to clear-up the coated tongue, cleanse the catarrhal stomach, stimulate the torpid liver, and facilitate the absorption of quinine like *calomel in large doses.* Give four compound cathartic pills (vegetable) or podophyllin 0.5 grain (0.032) if calomel can not be used.

2. If the patient presents a chill, fever, sweat, or complains of pain, administer phenacetine 5 grains (0.32) or aspirin 15 grains (1.0). This often shortens a paroxysm and brings on a sweat sooner than without these drugs. Repeat at end of two hours if symptoms indicate the need of it.

3. If patient is strong, and nausea is not troublesome, Warburg's tincture (with aloes) 0.5 ounce (15 cc.), repeated in three hours, is preferable to the aspirin or phenacetine. The drug should not be diluted or followed by other drink. The patient can remove the bitter taste by merely rinsing the mouth with water. The second dose of this remedy usually brings on a profuse perspiration after which all the symptoms of the attack rapidly disappear.

4. A full dose of magnesium sulphate or other saline should invariably be given within eight to twelve hours after the calomel.

5. Of course, ice bags and sponge temperature baths are very gratifying to the patient when the fever is troublesome.

6. Neo-arsphenamine (neo-salvarsan or "914") 0.45 to 0.75 gram intravenously as soon as convenient. It can be given at practically any time, even during the fever or sweating stages,

except when Warburg's tincture is being administered. It should then be given on the following day.

7. Ten grains (0.65) of quinine three times daily after meals for one week.

8. Thereafter 10 grains (0.65) of quinine daily, preferably after evening meal, for *seven weeks longer.* This is absolutely necessary to sterilize the patient of his infection and prevent relapses in the estivo-autumnal infestations, but should be the routine in all cases of malaria regardless of type.

If the patient neglects to take quinine daily, it would probably be easier for the physician to supervise this part of the treatment by having the quinine taken only on two successive days in the week. For convenience on Sundays and Mondays, when 10 grains (0.65) should be taken after each meal, a course of 60 grains (4.0) every week-end.

A great variety of individual symptoms naturally occur in marlaria. They usually are of fleeting character and disappear with the fever. If persistent they may be treated symptomatically as indicated. The loss of appetite complained of by nearly all malaria patients is but a symptom of the disease, and will correct itself upon overcoming the ague. Stomachic treatment is rarely necessary, as calomel and salts is the best treatment. In case the anorexia persists for a week or more the course of calomel may be repeated. Pain in the splenic area practically never requires special medication.

CHRONIC MALARIA

This is usually associated with relapses occurring at intervals which are multiples of a week (septenary periods), i.e., four, five or six weeks after acute attacks of malaria.

Treatment. Same as for acute malaria, with following suggestions: In long-standing cases with history of other relapses, (1) give injection of "914" 0.45 every week for two to six injections; (2) quinine 10 grains (0.65) daily, *plus* 30 grains daily on two successive days each week, *for two months.*

MALARIAL CACHEXIA

This state is characterized chiefly by severe anemia, lemon-colored complexion, weakness, loss of weight, enlarged spleen, often enlarged liver, and marked digestive disturbances.

Treatment. 1. The same as for chronic malaria.

2. It is important to add a good chalybeate tonic, such as Aiken's tonic, Blaud's Pill Improved, Four Chlorides, Elixir I. Q. &. S. Phosp., Syrup of Hypophosphites Comp., or tincture Ferric Chloride. The last mentioned is specially effective and may be conveniently given in ordinary gelatin capsules, prepared by the patient with aid of a medicine dropper immediately before taking, thus avoiding the astringent taste and damage to the teeth.

3. A mild saline purge every three or four days.

4. Ingestion of large quantities of water constantly.

Such a large percentage of malaria patients, particularly malarial cachetics, suffer with hookworms, ascarids, trichocephalae, and other intestinal parasites as a complication, that it is a good general rule to give a vermifuge in practically all cases. Thirty minims (2.0) of oil of chenopodium mixed in an ounce of castor oil and taken at a single dose, or one drachm, (4 cc.) of carbon tetrachloride in an ounce of water. Oil of chenopodium or carbon tetrachloride given in this simple manner usually does not require any subsequent purgation or dietary regulation, and they seem equally as safe and efficient as by any other method of administering these drugs.

PERNICIOUS MALARIA

The treatment of every case of pernicious malaria is heroic. There is no scene more spectacular in medical practice than the recovery of such a patient; it is one of the few instances in which a physician can truly feel that he has saved a life. The problem is to get into the victim's system a sufficient quantity of quinine, *without one minute of unnecessary* delay.

Comatose and delirious forms

Remember that the temperature may be normal or *subnormal* when these patients are first seen.

In the tropics every comatose or delirious patient who comes under the supervision of the physician should be regarded first as a victim of pernicious malaria. Sunstroke is exceedingly rare in the tropics, and comatose malaria is the diagnosis nine times out of ten. In coma with acute alcoholism, a history of injury—even a blow on the head— you can not go far wrong in applying the treatment outlined below, as very frequently the intoxication or the traumatism has merely flared up a latent malaria which produces the symptoms of coma. Too often patients have died in the tropics as a result of the physician's failure to recognize these facts.

Delirious cases should receive the same treatment.

Treatment. 1. Quinine hydrochloride, dihydrochloride or bisulphate, 15 grains (1.0) sterilized in 10 cc. or more of water, *by vein immediately.* Hurry!

2. Spinal puncture.

3. Calomel 5 grains (0.32) sprinkled under patient's tongue. Or, 1 drop of croton oil on back of tongue.

4. If temperature is subnormal, apply blankets and hot water bottles.

5. Quinine 15 grains (1.0) intramuscularly within one hour of the above intravenous injection, and every twelve hours thereafter until patient is able to take medicine by mouth.

6. Then begin treatment outline under "acute malaria" and continue as there directed.

Bilious pernicious malaria (vomiting type)

1. Calomel 5 grains (0.32). Calomel is soothing to the stomach and will often be retained when all other laxatives would be immediately rejected. One-half ounce (15.0) of sodium hypophosphate, a full dose of magnesium citrate, or other effervescent salts should be given, in divided doses, beginning eight hours after the calomel. If the saline is rejected, small

amounts should be continuously repeated at fifteen minute intervals until the patient has retained a full dose.

2. Due to the lessened coagulation time of the blood resulting from its large content of bile, and the fact there these patients are greatly weakened, intravenous injections are contraindicated. Intramuscular injections of quinine, 15 grains (1.0) in 10 cc. or more of solution, every twelve hours until fever is controlled are recommended.

3. The weakness of these patients makes it dangerous to use the stomach tube. If gastric lavage is desired it usually suffices to administer a couple of glasses of water, which will soon be vomited and thus cleanse the stomach.

4. To control the vomiting, morphine by hypodermic is efficient. Do not torture the weak sufferer with chloroform water, chloral, iodine, or other supposed gastric sedatives. Even if they might take the edge off the retching, they are dangerously depressing.

5. The vomiting in these cases, which is nature's effort at elimination, soon dehydrates the tissues, and it is a problem to supply such patients with a sufficient amount of fluids. Large quantities can be given by enema and by *subcutaneous injection.* Rectal feeding may be necessary where the vomiting continues several days.

6. When the fever is controlled, and the patient can retain medicines by mouth, administer an iron tonic as suggested for malarial cachexia; 30 grains (2.0) of quinine daily for one week; and, 10 grains (0.65) daily thereafter, at evening meal, for seven weeks longer.

7. When jaundice begins to disappear a rectal injection of "914" 0.9 will greatly aid convalescence.

Remittent ardent fever

Cases in which the temperature does not fall to normal, and often remains dangerously high.

Treatment. 1. Quinine 15 grains (1.0), in 10 cc. or more of solution, intravenously at once.

2. Neo-arsphenamine 0.9 by rectum.
3. Temperature baths at frequent intervals.
4. Within one hour of above intravenous injection of quinine, administer quinine 15 grains (1.0), in 10 cc. or more of solution, intramuscularly. Repeat every twelve hours until fever has been controlled.
5. Quinine 10 grains (0.65) by mouth, three times daily, beginning on afternoon of first day, and continuing for one full week after fever has been controlled.
6. On second day of treatment give calomel 5 grains (0.32), followed at end of eight to twelve hours by a saline purge.
7. Quinine 10 grains (0.65) daily, after evening meal, for seven weeks longer.

Other pernicious types

The four forms of malignant malaria described are those most frequently seen. Treatment for the other types does not differ materially from that of remittent ardent fever.

QUININE HYPODERMICALLY

No injection of quinine should be given in less than a 1:10 or greater dilution. The contents of the small quinine ampoules should always, if possible, be diluted with sterile water to the proper proportion. A 10 cc. Lüer syringe filled to capacity will hold enough solution for an injection of 15 grains (1.0) of quinine. Due to the almost certain tendency to produce a slough quinine ought never be given subcutaneously. Intramuscular injection is the method of choice, the injections being made into the gluteal muscles 1 or 2 inches below the iliac crest. Rigid asepsis is imperative. Quinine in concentrations of 1.5 per cent are anesthetic, and a dilution of 15 grains (1.0) in 10 cc. does not need the addition of novocain, urethan, or other local anesthetics, to prevent pain.

In spite of all precautions indurations and abscesses sometimes result, although but rarely when a 1:10 or higher dilution is employed. A 30 per cent ichthyol ointment and a hot water bottle if applied early will usually abort an abscess. A short rubber tube connection between the needle and syringe is of great service in preventing a struggling patient from breaking off the needle during the injection.

ARSPHENAMINES IN MALARIA

The employment of the arsphenamines in malaria has received but passing attention, most writers only referring to it as a last resort when quinine fails, to be used in the later or chronic stages of the disease. Some men of authority do not even mention it in their works. We desire to call particular attention to these drugs in the *early treatment of malaria,* as it has been constantly demonstrated that they will abort the symptoms in practically every attack of malarial fever. The principal value of neo-arsphenamine is that it immediately cuts short the tertian, quartan or estivo-autumnal seizures before the repeated fever can have time to severely debilitate the patient, thus his convalescence is markedly shortened and the chances of recovery correspondingly enhanced.

There usually succeed no other paroxysms after the injection; although only one more single attack may occur the same or following day. Reactions from "914," when given in concentrated solution, are very few in malaria—the chill, fever and sweat which rarely follow within a few hours constitute merely the typical malaria picture which has been precipitated by the injection, a sort of malarial Herxheimer. It would have come tomorrow anyway.

The utility of "914" in malaria, then, is (*a*) it stops the attacks promptly before the patient is greatly weakened by the fever and plasmodial toxins, (*b*) through its excellent tonic value it rapidly builds up the patient, (*c*) patients recover their strength quickly and return to work considerably sooner than with quinine treatment alone, (*b*), it is effective when even quinine in enormous doses occasionally fails, i.e., where quinine-fast organisms are present, or for some reason the quinine is not properly assimilated.

Neo-arsphenamine alone is specific in tertian malaria. Estivo-autumnal constitutes 50 per cent of all malaria in the river valleys of Colombia, and this type requires quinine in addition to "914," but in much smaller quantities than would be necessary without the arsenical. As estivo-autumnal (sub-tertian) may very closely simulate true tertian malaria, presenting quotidian or tertian recurrences, it is advisable to combine "914" and quinine in all cases of malaria regardless of type.

Malaria is often complicated by other constitutional diseases, such as yaws, syphilis, trypanosomiasis, filariasis, uncinariasis, amebiasis, etc., which usually are greatly aggravated by the fever, and may quickly carry a patient to his death. As "914" has a specific effect in the first three and is a distinct help in combating the anemia of the other diseases mentioned, its routine use in malaria will correct a large number of complications arising from other sources.

American relapsing fever (the spirochetal fever of Dutton and Novy) is increasing in frequency. The clinical symptoms at the beginning resemble malaria so that it is very readily confused with the latter. Quinine has practically no effect upon relapsing fever, but the arsphenamines are specific, so that their use as a routine in malaria also "catches" the cases of relapsing fever.

All brands of neo-arsphenamine, American and foreign, have been given a thorough trial in our medical service, and we are partial to the product of the Dermatological Research Institute, Philadelphia. It is rapidly soluble (very important where many injections are given in a busy clinic), makes a beautifully clear solution, is remarkably free from anaphylactic or nitritoid reactions, and gives distinctive clinical results in malaria. Each ampoule is packed in an individual sealed wooden box which eliminates breakage, but more important still protects the drug from rapid temperature changes (wood being a poor conductor of heat) and thus reduces almost to zero the number of tubes which so frequently are prone to go bad. Only physicians living in warm climates who have had a large percentage of organic arsenicals damaged by the heat, with the consequent financial loss, and the very grave danger to their patients in the use of a partially deteriorated drug, can fully appreciate the genuine value of the above apparently insignificent matter of packing. In the tropics, all supplies of "914" or "606" should be kept in a refrigerator, but such advantages are available to only a relatively small number of tropical practitioners.

Intravenous method. Contraindications: Albuminuria, severe jaundice, organic heart disease, marked anemia or debility, idiosyncrasy to arsenic.

1. Sterilize syringes (2 cc. size) by boiling in filtered river water for two minutes. Double-distilled water is good but not necessary where river, rain, or other non-impregnated water is available.

2. Pour off all water but an ounce or two and set boiler in a basin of cold water. This will quickly cool the syringes and the sterilized water to room temperatre. Hot water should never be used for dissolving the "914."

3. Sterilize the points of a hemostat or other forceps by dipping in alcohol, or passing through a flame. With forceps take out (*a*) barrel of syringe; (*b*) then needle, attaching it to barrel; (*c*) next plunger of syringe, inserting into barrel. All of this can be done with fingers sterilized in alcohol.

4. Draw into the syringe 2 cc. of the water in which the syringes were sterilized.

5. File off the small tip of the neo-arsphenamine ampoule.

6. Inject the 2 cc. of water into the ampoule. Dissolve the "914" by drawing the solution back into the syringe and reinjecting it into the ampoule several times, or better by sterilizing the tip of the index finger in alcohol, clamping it over the opening in the ampoule and shaking. This dissolves the drug at once, and replaces the air-tight, glass-stoppered bottle formerly used for effecting the solution.

7. Draw up required dose of solution in the syringe. Next fill the syringe completely by drawing in more sterilized water.

8. Sterilize site over vein with tincture of iodine.

9. Have an assistant, or the patient himself, grip the arm tightly with the hand above site of injection (no need to bother with a tourniquet). When vein is distended, insert needle (with bevel on under side), watch for column of blood to rise in the syringe, indicating that the needle is in lumen of the vein. Release grip on arm, and begin injection.

10. Inject very slowly, *being extremely careful that none of the solution gets into the tissues outside the vein.* It will cause severe pain and indurations. Should this happen, give the patient a dose of codeine or morphine to relieve his discomfort, and do not irritate the painful area with local applications. The indurated spot will be absorbed within a couple of weeks.

11. If the plunger should stick in the syringe, twist it to loosen. If at any time in doubt as to whether point of needle is in the lumen of the vein, slightly withdraw the plunger. A column of blood appearing in the syringe will set you right. If no blood appears upon careful backward manipulation of the plunger, withdraw needle, eject a few drops of the solution to clear it out, and begin injection in another vein.

The little syringe filtering adapter for administration of neoarsphenamine, described by Dr. Schamberg (Jour. Amer. Med. Assoc., July 15, 1922, p. 216 Becton-Dickinson & Co., Rutherford, N. J.) is an excellent improvement which re-filters the solution while being injected.

While it may appear that the operation just described does not show the necessary refinement of the intravenous method, yet it has been proved in over 3000 injections given in this medical service that there is probably no more practical, efficient, or less dangerous way of administering "914," and we would not consider returning to the elaborate artillery in common use.

The advantages claimed for the method is that it is very simple, requires but little time, and renders the application of neoarsphenamine possible in practically any case, a thing of real importance to the general practitioner. With the little pocket sterilizing hypodermic outfit marketed by Becton-Dickinson & Co. the injections may be given anywhere and under almost any circumstances. This means that "914" can thus be made available to the greater number of people who suffer with malaria, the country population

One of the practical advantages of the small syringe and needle is that the injections are almost painless and patients do not resent them any more than other hypodermic medication; many submitting when otherwise it would be difficult or impossible to get them to take it. With the small needle it is frequently possible to enter a very small vein, which could not be done with the large needles or cannulas usually employed in arsenical injections. It is never necessary, even in children, to dissect out a vein for an injection.

Rectal method. Neo-arsphenamine administered in this manner is not attended with bowel irritation, reactions, or other uncomfortable symptoms, and there seems to be practically no contraindications to its use by rectum, except possibly arsenic idiosyncrasy. Apparently it equals the intravenous method in efficiency, but is somewhat slower in action, requiring two or three days to check the fever. The rectal dose is double the intravenous dose.

1. Boil 4 ounces (128 cc.) of water and allow to cool until lukewarm.
2. Dissolve in the water neo-arsphenamine 0.9 gram.
3. Inject in rectum, with or without previous cleansing enema.
4. Require patient to remain in reclining position from six to eight hours.

OTHER DRUGS IN MALARIA

Quinine is the king of antiperiodics, but in persons who suffer from quinine allergy, quinine neuritis (particularly optic neuritis), or who are greatly nauseated by the drug, it may be advisable to try some of the remedies presently to be described. It is well to remember that 90 out of every 100 people who say they cannot take quinine, can do so if the drug is disguised. However, there are some persons who can be severely poisoned by even 1 grain (0.065) of the drug, and in whom all desensitizing methods completely fail to increase their tolerance. It would be malpractice to try to force quinine upon them.

When there are heart disorders, quinine intolerance, or the person is so weak as to render intravenous injections of ordinary quinine salts inadvisable, it is possible to use "Colobiases de Quinine," a colloidal quinine (Laboratories Pharmaceutiques Dausse, Paris), in the dose of 0.5 grain (0.033) in 2 cc. of a 10 per cent suspension of gum acacia, intravenously. It gives satisfactory results in all forms of malaria. It should be obtainable in ampoules of proper dosage at any pharmaceutical jobbing house.

In conjunction with the above, it is of value to administer a course of 6 hypodermic injections of 10 cc. each day of "Diéménal," colloidal manganese and iron, (Laboratories de Diéménal, Fontenay-s-Bois, près Paris), which is decidedly antiperiodic, but has its chief value in its rapid tonic effect. It is very useful in severly debilitated patients.

Where a substitute for oral quinine is desired, selection may be made from the following, each of which we have found efficient:

15 grains euquinine (quinine ethyl-carbonate) = 8 grains quinine sulphate
15 grains methylene blue = 25 grains quinine sulphate
15 mm. oil of eucalyptus, or eucalyptol = 10 grains quinine sulphate
60 mm. Warburg's tincture = 5 grains quinine sulphate

A very pure product of methylene blue must be used, accompanied by large quantities of water, and if the drug shows a tendency to produce strangury equal parts of powdered nutmeg should be added. It is advisable to give equal parts of sodium bicarbonate with each dose of oil of eucalyptus or eucalyptol. Most drug houses put on the market Warburg's tincture in tablet form, a tablet representing 1 drachm of the solution. While these are efficacious and convenient, yet the solution is probably more active.

Splenic extract has been extolled in malaria. After giving it a thorough clinical trial we failed to note any antiperiodic value from it, even the results of extremely large doses of a fresh product being almost nil. Possibly there resulted a transient tonic effect through the nucleins it contained.

Many people of the malarial districts, especially in Spanish-America, have little faith in quinine. During their attacks of malaria they have been accustomed to take only enough of the drug to relieve the acute symptoms without continuing its use until they were sterilized of the plasmodia, and as a consequence have suffered repeated relapses. Commonly they will not take quinine for malaria, but are strong believers in "tonics for the liver," readily accepting almost any liquid medicine recommended to them as such. The following formulas have been used in our practice with success, many of the people even using daily doses as a prophylactic:

℞

Tr. antiperiodica sine aloe (N.F.)	f℥xvi	1000.0 cc.
Quininae hydrochloridi, vel bisulphatis	ʒxv-M.	60.0

Sig. Each teaspoonful contains therapeutic equivalent of 5 grains (0.32) of quinine.

Or

℞

Quininae hydrochloridi, vel bisulphatis		ʒ xxi	84.0
Strychininae sulphatis		gr. iii	0.2
Glycerini		f℥ vi	180.0
Aquae	q.s. ad.	0 ii	1000.0—M.

Sig. Each teaspoonful contains approximately
5 grains (0.32) of quinine.

If desired syrup of yerba santa or syrup of chocolate may be added to the above, particularly in administering quinine to children. Some attractive coloring is of psychical advantage when stock solutions are used in dispensary practice.

SUBSTITUTES FOR NEO-ARSPHENAMINE

If inadvisable for any reason, except idiosyncrasy to arsenic, to administer "914" by vein, it may be substituted by sodium cacodylate 5 grains (0.32) or di-sodium methylarsenate (arrhenal) 5 grains (0.32). Either of these may be dissolved in 10 cc. of water, sterilized, and given intravenously or intramuscularly. The dosage may appear to be large, but large doses of all drugs are required in malaria. We have seen no reactions or bad effects from such quantities. An injection on two successive days each week, for two weeks in acute malaria; for five weeks in chronic malaria.

Sulpharsenamine (Sulfarsenol), 0.3 gram to 0.5 gram, subcutaneously or intramuscularly, is convenient and satisfactory. It has relatively the same value as neo-arsphenamine given by rectum. It must be given with much care in algid malaria due to the deficient superficial circulation.

If injections of any kind are impracticable, saturate the patient quickly with Fowler's solution or arsenious acid, observing the usual precautions.

Some doubt has been expressed by certain writers as to the value of arsenic in malaria. Without going into theoretical discussion, we wish to express our absolute faith in the genuine clinical value of the drug, as demonstrated in our practice on hundreds of occasions.

QUININE PROPHYLAXIS

To prevent infection with malaria is possible in the great majority of cases, provided a sufficient dose of the drug is taken. Practically all authorities in the tropics now agree that 10 grains (0.65) of quinine daily is the required amount. A lesser quantity, while giving a certain measure of protection, is not adequate. We have seen no ill results from the above amount daily over long periods of time.

RELAPSES

It is our practice to forbid alcohol in any form to persons suffering with malaria. We recognize the excellent value of alcohol properly used in fevers due to pneumonia, tuberculosis, influenza and sepsis, but are confident that it is all wrong in malaria. We have often seen acute paroxysms of fever precipitated in latent malaria by very ordinary quantities of alcoholics. The old popular "whisky and quinine" prescription produced more chronic cases of malaria than it ever cured. The small amount of alcohol in Warburg's tincture can be classed as a necessary evil.

If possible to prevent it, malaria patients should never be given iodides, adrenalin, serums or vaccines, treated with ultra-violet rays or therapeutic lamps, and should avoid the sun light, as all these are peculiarly prone to produce relapses. Excessive heat of any kind is contraindicated—malarial relapses among cooks, firemen, laundry workers, and the like, being notoriously frequent.

Cold or prolonged baths are to be avoided, except during the fever stages, until the patient is thoroughly convalesced.

Many patients with chronic malaria or severe acute malaria can only be cured in bed. Rest is a most important part of the treatment in every case of the disease. The patient who is up and around throws off a great part of the effective value of the drugs he is taking.

Do not be too quick to decide that a patient requires to go to a colder climate or to the seashore to be cured. While this is always desirable, if practicable, yet by following faithfully the line of treatment given in the preceding pages, and demanding that your patient rest at home just the same as he would on a vacation elsewhere, every case of malaria can be cured right in the hot lands. We have seen dozens of patients, even among North Americans and Europeans, suffering with chronic or pernicious malaria, recover completely and show a gain in weight over that enjoyed upon arrival in the torrid areas of the tropics. The invaliding rate for the above class, once a serious problem for our medical department, has been reduced to practically zero. In fact, in this most pestilential of regions, malaria is but little feared now. Neo-arsphenamine, applied early in all instances, and, if necessary, throughout the course of the chronic cases, has largely made this possible.

SUMMARY

1. The immediate problem of malaria is one of therapeutics rather than sanitation.
2. The accepted death rate of 2.89 per cent in ordinary malaria, and 26.6 in pernicious malaria, deserves serious attention.
3. In a series of 8000 cases ocurring in tropical practice the mortality was reduced to 0.2 per cent for general malaria, and to 6 per cent for the malignant types.
4. The arsphenamines promptly abort acute attacks in all forms of malaria, and are specifics in the tertian field.
5. Neo-arsphenamine in concentrated solutions—as much as 0.9 gram in 2 cc.—fulfills all essential requirements of the higher dilutions. Technic of injection is given.
6. Neo-arsphenamine by rectum is efficacious.
7. Colloidal quinine (Colobiases de Quinine—Dausse), in doses of 1 grain (0.065), is therapeutically equivalent to 15 grains (1.0) of ordinary quinine salts, and is non-toxic.
8. Colloidal manganese and iron (Diéménal) is antiperiodic and reconstructive.
9. The management of acute, chronic, cachectic and pernicious malaria is outlined.

SUMARIO

1. El problema inmediato de la malaria es mas bien uno de terapéutica que de ciencia sanitaria.

2. La proporción aceptada de mortalidad de 2.89 por ciento en la malaria corriente y de 26.6 en la perniciosa, merece la más seria atención.

3. En una serie de 8,000 casos ocurridos durante el ejercicio profesional en los trópicos, la mortalidad de la malaria general fué reducida a 0.2 del 1 % y a un 6% la de los tipos malignos.

4. Las arsenofenaminas abortaron con prontitud los ataques agudos de todas las formas de malaria y son específicos en el campo de las tercianas.

5. La neo-arsenofenamina en solución concentrada, o sea de 0.9 gramos en 2 cc., posee todos los requisitos esenciales de las mayores diluciones. Se explica la técnica de inyección.

6. La aplicación de neo-arsenofenamina por el recto, es eficaz.

7. La quinina coloidal (Colobiases de Quinine—Dausse), en dosis de 1 grano (0.065), es terapeuticamente equivalente a 15 granos (1.0) de las sales de quinina corriente y no es tóxica.

8. El manganeso y el hierro coloidal (Diemenal) es antiperiódico y reconstructivo.

9. Se reseña la dirección del tratamiento de casos agudos, crónicos, caquécticos y perniciosos.

GRANULOMA INGUINALE

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In the recent literature[1] cases of granuloma inguinale have been reported from widely separated areas. To this list I wish to add 2 cases from Columbus, Georgia.

Case 1. T. C., female, white, age twenty-four, widow, admitted to the City Hospital, August 2, 1922, complaining of a very painful ulceration in the right gluteal fold. She was born and lived in southern Georgia. Had one child, no miscarriages. The new born was "covered with pus pockets" and died in the sixth month.

In 1918 she fell astride the bed striking her right groin. This was followed by a painful swelling which developed into an abscess requiring a lancing a week later. Instead of healing, the wound became ulcerated. A curettement of the ulcer three months later was without success. It crept slowly over the pubis to the left groin and thigh, thence in the hair line of the labia majora to the perineum, around the anus to the ischiorectal fossae. The ulcer was extremely painful, bled readily, and had an offensive odor. Improvement would usually be followed by breaking down. In January, 1922, without a particular cause, the ulcer began to heal, and was entirely well during February and March. In the latter part of March she fell and cut herself on the right gluteal fold. The wound did not respond to treatment, but became ulcerated and behaved similarly to the ulcer described above.

Following the ulceration of the left groin and thigh in 1920, the left labia minora began to enlarge. The enlargement never has been painful or ulcerating.

Since the appearance of the ulcer in 1918, she has received over forty salvarsan treatments, besides more or less iodide medication. One

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[1] Complete bibliography is given by the authors listed in reference list at end of article.

Wassermann test in 1921 has been reported positive out of a number of others reported negative.

Examination shows moderately enlarged cervical, axillary and inguinal lymph glands. Epitrochlear glands not palpable. Pupils equal, regular, react to light and accommodation. Spleen and liver not palpable. Somatic reflexes present and normal. Romberg absent. Patient is unable to extend her right leg without excrutiating pain. There is a dense scar tissue present over the right groin, the pubis, the left groin and thigh, the perineum, and both ischiorectal fossae. An ulcer 7 inches by four extends from the lower right edge of the symphysis pubis along the right gluteal fold. The border is slightly raised, not pearly, not inflamed, not indurated, except where healing has taken place. The edge is sharply demarcated and in places shows a slight undetermining, but without sinuses formation, tubercles or purulent discharge. The base is irregular, soft, puckering; bleeds profusely; is covered with a slimy serous discharge of extremely

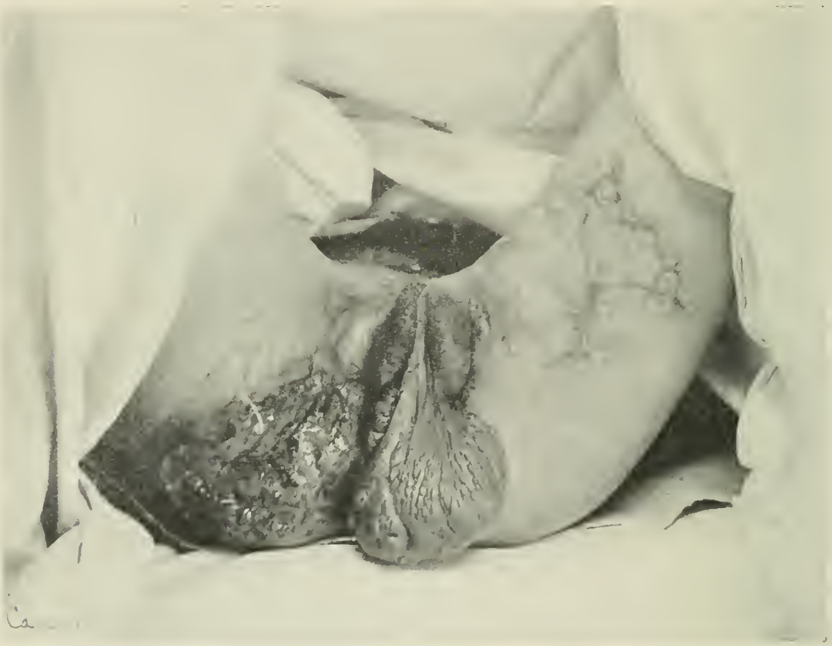

Fig. 1. Case 1. Ulcer of Five Months Duration

disagreeable odor. The ulcer is very painful to touch. It is serpiginous in nature. The healed portion is hard and painless.

Laboratory findings show negative Wassermann and Kahn's precipitin reactions on blood as well as spinal fluid. Globulin and cell count of spinal fluid were in normal limits. White cells 7400, red cells 3,800,000; neutrophils 62 per cent, lymphocytes 28 per cent, eosinophils 10 per cent. The stained preparation from the ulcer showed a large number of eosinophils and an almost pure culture of diphtheroid organisms, which showed a considerable resemblance to Klebs-Loeffler bacilli. The tissue from the base of the ulcer showed a very vascular fibrous tissue, diffusely infiltered with round cells. Repeated examination of stained smears failed to show the Donovan bodies.

Diagnosis. Granuloma inguinale with a secondary diphtheroid infection.

Irrespective of the failure to demonstrate the Donovan bodies microscopically, intravenous treatment with 1 per cent tartar emetic in a sterile physiological saline solution was instituted. In ten days there was considerable improvement. The patient became less irritable and developed a ravenous appetite. The repugnant odor disappeared, the bleeding stopped and the ulcer could be dressed without an analgesic. However, the ulcer was healing slowly. Then ten thousand diphtheria anti-toxin units were injected, as it was suspected that the persistence of the diphtheroid organisms might have something to do with the slow progress. Following the anti-toxin administration, the ulcer was healing rapidly and had diminished more than half in size, when the patient contracted rhinitis and a slight pharyngitis, and with this the new formed skin melted away in less than twenty-four hours. Thus at the end of six weeks the size of the ulcer was the same as before the commencement of the treatment, though the general condition of the patient was very good. As soon as the rhinitis cleared up, new skin began to proliferate very rapidly and at the end of the ninth week the ulcer was about half way healed. Unfortunately, the patient then left the hospital. Five weeks later she came back. The ulcer was now about the size of a silver dollar, entirely superficial, in perfect condition of healing, absolutely clean and covered with a healthy granulation tissue. Two intravenous injections of tartar emetic were given on two successive days, after which she entirely dissappeared. During the five weeks while she was gone, she had four more salvarsan treatments.

Case 2. J. B., male, negro, age twenty-seven, single, life convict, referred by Dr. Cosby, November, 1922, for diagnosis and treatment of a long standing ulcer over the pubis and right groin.

Family and past history unimportant. Present illness began seven months ago. A hard, painful nodule appeared over the symphysis at the base of the penis, two weeks following coitus with a habitual prostitute. The nodule was painful at the start. He attempted to "pick it off." It soon became ulcerated and gradually spread in the line along the pubis and the base of the scrotum in the right groin. The ulcer never has been purulent. It discharged copiously a yellowish clear serum of repugnant odor. It bled readily and has been painful to touch.

Physical examination reveals a generalized adenitis, including large epitrochlear glands. Pupils equal, irregular, react to light and accommodation. Somatic reflexes present and normal. Romberg absent. The ulcer extends in the form of a semilunar ring around the base of the penis and the scrotum on the right side. It is $\frac{3}{4}$ inch wide and about 4 inches long. The border is rough, raised, indurated. The edge is sloping towards a shallow base, which in its upper third portion shows ulceration through the skin. In the lower half of the lesion, the corneum is gone, leaving behind the deeper layers of the dermis, with two pea sized areas extending through the epithelial layers. However, both the ulcerated as well as the nonulcerated areas are painful to touch, and the slimy yellowish clear, repugnant secretions appear through the lesion. Bleeding is present only in the ulcerated portions.

Laboratory findings show negative Wassermann and Kahn's precipitin reactions. White cells 14,000, neutrophils 88 per cent, lymphocytes 6 per cent, transitionals 5 per cent. Smears from the lesions besides showing numerous Donovan bodies, revealed rich culture of the fusiform bacillus and the spirochetae of the Vincent's angina.

Diagnosis. Granuloma inguinale, Vincent's angina.

Treatment. As some of the granuloma cases improve with difficulty under treatment, it occurred to me to study the effect of tartar emetic upon Vincent's angina before administering the specific remedy for the latter condition. With this in view, nothing but tartar emetic was given intravenously for eighteen days. Marked constitutional improvement followed the treatment. Locally pain and odor disappeared, but the appearance of the ulcer remained the same. Granuloma bodies were not found in the stained smears after the second treatment with 0.04 gram of tartar emetic. The organisms of the Vincent's angina remained as abundant at the eighteenth day, after administration of 0.80 gram of tartar emetic, as before the commencement of the treatment. On the eighteenth day 0.40 gram of salvarsan was administered. On the following day the serous discharge had stopped and the ulcer had dried up considerably. The organisms of Vincent's angina were still abundant on the moist surface between the scrotum and the leg and disappeared only as rapidly as the skin dried up. On the sixth day following the salvarsan administration the ulcer was completely healed and no fusiform bacilli or spirochetae were found in the scrapings from the healed area. At this time the blood picture showed white cells 5300 neutrophils 60 per cent, lymphocytes 31 per cent, eosinophils 4 per cent, transitionals 4 per cent and basophils 1 per cent.

COMMENT

In both cases success of specific treatment depended considerably upon the measures taken to control the secondary infection; in the first case a diphtheroid, in the second case the organisms of Vincent's angina. In the series reported by Randall, Small, and Belk (1) they mentioned the presence of the diphtheroid organisms in several of their most obstinate cases. In my first case, as long as the diphtheroid organisms were present, the lesion improved very slowly. Only after combined treatment with the tartar emetic, diphtheria antitoxin and iodoform powder, the diphtheroid organisms disappeared and the wound showed rapid progress. In my second case, no improvement appeared in the lesion from tartar emetic administration until specific treatment for the secondary invading organisms of Vincent's angina was carried out.

SUMMARY

1. Granuloma inguinale is undoubtedly common in middle and southern Georgia.
2. A secondary infection may delay and prevent the benefit of the specific treatment by tartar emetic.
3. The organisms of Vincent's angina are not affected by tartar emetic.
4. To hasten the healing of the granuloma inguinale lesions, the secondary infection must be treated with its specific remedies along with the treatment for granuloma inguinale.

In conclusion the writer wishes to express his thanks to Drs. Bert Tillery and V. D. Stone for their valuable suggestions and assistance.

RESÚMEN

El autor dá amplios detalles acerca de dos casos de granuloma inguinal que tuvo bajo observación y tratamiento en el Estado de Georgia (E.U.A.) y hace el siguiente comento:

El éxito del tratamiento específico de ambos casos dependió considerablemente de las medidas tomadas para controlar la infección secundaria. Uno de los casos presentaba una infección secundaria difteroide; el otro, de organismos de la angina de Vincent. En la serie de casos ya publicados por Randall, Small y Belk (1) se menciona la presencia de organismos difteroides en los más obstinados. En mi caso, mientras dichos organismos estuvieron presentes, la lesión mejoró muy lentamente. Solamente despues del tratamiento combinado con tártaro emético, antitoxina diftérica y yodoformo en polvo pudieron aquellos desaparecer y notarse progreso en la curación. En el otro caso no se notó me joría mediante la administración del tártaro emético hasta que no se instituyó tratamiento específico en contra de los organismos de Vincent. Hace las siguientes conclusiones:

1. El granuloma inguinal es sin duda común en la parte central y meridional de Georgia (E. U. A.).

2. La infección secundaria retarda y evita el beneficio del tratamiento específico con el tártaro emético.

3. Los organismos de Vincent no son afectados por el tártaro emético.

4. Para acelerar la curación del granuloma inguinal es necesario que las infecciones secundarias sean atacadas con remedios específicos en combinación con el tratamiento especial de la granuloma inguinal.

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METHODS OF ADMINISTERING ANTHELMINTICS TO REMOVE WHIPWORMS WITH A NOTE ON A NEW METHOD

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In the present state of our knowledge, the removal of whipworms by anthelmintics is a difficult matter. There is so little certainty of drug action in this respect, so far as the administration of one or two doses of most anthelmintics is concerned, that Berard and Vignard (1) reach the conclusion that it is necessary to resect the appendix when these worms are present in the appendix, and in veterinary practice, Miller (2) comes to a somewhat similar conclusion in regard to whipworms in the cecum of dogs and reports favorably on resection of the cecum as a cure for canine trichuriasis. In this connection it may be noted that Matsuoka (3) found that whipworms were very seldom found in the appendix, although whipworm eggs were often found there, contrary to the case for pinworms which are often found in the appendix, although their eggs are rarely found there. The absence of eggs is associated, of course, with the fact that the gravid female pinworm does not deposit eggs until after she has left the body of the host.

In the view of the fact that a major operation is too elaborate and expensive a procedure to warrant resort to it for a condition such as trichuriasis, which in most cases does not give rise to a serious pathological condition, the possibilities of dependable anthelmintic medication must receive more attention before we can conclude that medicinal treatment promises nothing and that operative interference is the only resort. Numerous tests of anthelmintics on dogs show that many drugs, even comparatively feeble ones, will occasionally remove some or all of the whipworms present in these animals, though as a rule these treatments fail to remove worms. The conclusion arrived at by Hall was expressed by him (4) as follows:

> The difficulty is not due to any resistance to vermifuge action on the part of the worm, but to the likelihood that vermifuges, after reaching the ileocecal valve, will be carried down the colon without entering the cecum and coming into contact with the whipworms present. It therefore appears that any treatment directed against this worm will depend for its efficacy on the factors which determine whether the medicine employed will pass into the cecum, and unfortunately we do not appear to be aware of the nature of these factors or how to control them. It may be found good practice to administer anthelmintics in small doses over a long period of time to ensure the entrance of the drug into the cecum. Wade reports success with 2-grain doses of thymol three times a day over a period of two weeks.

Hall (5) found that repeated doses of chenopodium displayed an increasing degree of efficacy against whipworms in dogs with an increasing number of daily doses of the drug, but found this drug too irritating to the digestive tract and recommended santonin; subsequently he (6) published experiments with repeated doses of santonin, showing that this method of treatment gave fairly satisfactory results.

Another evident method of attack on whipworms would be to use some comparatively non-toxic anthelmintic which could be given in such large doses as to ensure the entry of the drug into the cecum with some degree of certainty. Apparently the latex of the fig, *Ficus laurifolia*, a native of South and Central America, is such a drug. This drug has been recommended for this purpose by Berrio, Mouatt-Biggs, Henao and Villa, Lezaca, Montoya and others. The drug is given in doses of 10 to 45 grams, and in all probability its bulk is a determining factor in its reported efficacy. Unfortunately, according to Balfour (7), the latex ferments readily and only keeps four or five days under ordinary conditions, although it will keep in an icebox for thirty-four days. At present it can only be used to advantage in countries where the tree grows. Nor is the drug entirely non-toxic and free from danger to patients. Montoya

(8) reports that it may cause, in some cases, cramp, delirium, sudden and intolerable urticaria, syncope, vertigo, convulsions, rectal and vesical tenesmus, partial suppression of urine, etc., and the writer has personal knowledge of one death from this drug.

Aside from operation, repeated small doses of a non-irritant anthelmintic, and large doses of a relatively non-toxic anthelmintic, another method of attack for whipworms has been tested by Strong (9), who states:

> Besides giving vermifuges in the usual manner, I have also tried intramuscular injections of thymol in sterile olive oil and also chenopodium in the same manner, but without any noticeable change in the number of eggs appearing in the stool, and also careful examination of all stools, over a period of a week while on daily injection, failed to show one whipworm.

This is the only published report known to the present writer of a test of intramuscular injections of anthelmintics for the removal of whipworms.

INTRAMUSCULAR INJECTION OF OIL OF CHENOPODIUM

It has always been assumed that oil of chenopodium acts on hookworms by direct contact with the parasites in the intestines. Recently the suggestion was made that the effect of the drug might result, entirely or in part, from its absorption through the intestinal walls into the circulation, and its subsequent ingestion by the worm by mouth. It was thought that if this were true, good results should be obtained by injecting suitable doses of the oil intramuscularly. For use in this way oil of chenopodium was prepared by mixing it with camphorated oil and resorcin according to Heiser's formula for preparing chaulmoogra oil for intramuscular injection for leprosy. The formula, as used, follows: 60 cc. oil of chenopodium, 60 cc. camphorated oil, 4 grams resorcin. The tests here described were made in the course of a hookworm infection survey of New Guinea, conducted between March 18 and October 26, 1921.

Case 1. In the morning 4 cc. of this mixture, the equivalent of 2 cc. of oil of chenopodium, were injected intramuscularly into the buttock of one native. Magnesium sulphate was given in the afternoon and again each day for five days. Six days' stools were examined for worms. None was found the first three days, 1 hookworm was found on the fourth day, 3 hookworms on the fifth day, no worms on the sixth; the total number of parasites recovered was 4 hookworms. The test treatment expelled 89 hookworms, 1 Ascaris, and 1 Trichuris.

Case 2. To a second patient 10 cc. of the same mixture, equivalent to 5 cc. of oil of chenopodium, were given intramuscularly. The purgative was administered as in case 1. Stools were examined for six days. On the second day 2 hookworms were expelled, and on the third day 1 Ascaris. No other worms were recovered. After the test treatment 155 hookworms and 1 Ascaris were expelled.

Case 3. In the case of a third patient 3 cc. of oil of chenopodium, undiluted, were injected into the buttock. The purgative was given as in cases 1 and 2, and the stools were examined for six days. Worms were recovered as shown below.

Worms recovered after injecting 3 cc. oil of chenopodium intramuscularly

	HOOKWORMS	OXYURIS VERMICULARIS	TRICHURIS TRICHIURA	ASCARIS LUMBRICOIDES
First day	0	9	2	0
Second day	0	0	9	2
Third day	0	0	0	0
Fourth day	0	0	11	1
Fifth day	0	0	0	0
Sixth day	0	0	0	0
Total	0	9	22	3

No test treatment was given to this patient as the experiment was made in the last two weeks of the campaign and there was not enough time. Examination of his stools had shown the presence of hookworm ova.

In all 3 patients there were no local signs or symptoms at the site of injection. The first patient, case 1, showed no symptoms whatever. A curious feature was that a severe intractable local skin disease, otherwise untreated at this time cleared up rapidly in the four weeks following the treatment. The second patient, case 2, showed the typical symptoms of absorption of oil of chenopodium, indicating that the dose was excessive. The third patient, case 3, showed no symptoms of any description.

The following conclusions may be drawn from the results of these intramuscular injections of oil of chenopodium:

1. Oil of chenopodium, injected intramuscularly, has little vermicidal action on hookworms.

2. It apparently had in case 3 a marked vermicidal action on *Trichuris trichiura*, the whipworm.

The largest number of Trichuris expelled by any of the numerous persons in New Guinea, who, during the recent hookworm infection survey, received chenopodium treatment by mouth, was 15, following a dosage of 3 cc. of the vermifuge. Yet 22 Trichuris were removed in case 3 following the injection of 3 cc. of oil of chenopodium intramuscularly.

THE INTRAVENOUS INJECTION OF OIL OF CHENOPODIUM

After the tests in which oil of chenopodium was injected intramuscularly, with the result that Trichuris, in one of the tests, were expelled in considerable numbers, it was decided if possible to see if a better result could be obtained by the intravenous administration of the drug. In recent years, intravenous therapy has advanced rapidly and many metallic and other preparations are now used intravenously which would formerly have been considered inadmissible. With the coöperation of the local physicians, an opportunity was afforded to try this kind of treatment three times.

Case 1. One patient was given 2 cc. of oil of chenopodium intravenously. Four hours later, and daily during the next four days, 2 ounces of castor oil were given. The results of worm counts during five days after treatment were as follows:

Results from 2 cc. oil of chenopodium administered intravenously

	HOOKWORMS	OXYURIS VERMICULARIS	TRICHURIS TRICHIURA
First day	0	356	0
Second day	0	526	3
Third day	0	0	8
Fourth day	0	0	0
Fifth day	0	0	0
Total	0	882	11

The oil of chenopodium was injected slowly at 10 a.m. The patient seemed to suffer a momentary syncope. His pulse rate dropped from 72 to 60. He vomited and had hiccough, but at 11 a.m. he seemed much better and continually wanted to sit up. At 10 p.m. his pulse rate was 66. At 6 a.m., the first day after the injection, his pulse was normal. A test treatment of 2 cc. of oil of chenopodium given one week after the injection, removed 33 hookworms and 1 Trichuris. The patient had no noteworthy symptoms at this time.

Cases 2 and 3. The other two patients each received 1.5 cc. of oil of chenopodium intravenously at the same time. Their clinical histories were similar. Immediately on receiving the injection they had syncope which lasted for a few moments. The pulse rate dropped to 60. Forty-five minutes after the injection they vomited. After that they apparently felt quite well and were able to stand and walk about. By 5 p.m. the pulse rate was normal. Castor oil was given daily as in case 1, and the stools were examined for worms.

The results of the tests are combined in the following table:

Results from 1.5 cc. oil of chenopodium administered intravenously

	CASE 2			CASE 3		
	Hookworms	Trichuris	Ascaris	Hookworms	Trichuris	Ascaris
First day	0	3	1	0	1	0
Second day	0	12	1	0	22	2
Third day	0	0	0	0	7	0
Fourth day	0	4	0	0	0	0
Fifth day	0	0	0	0	0	0
Sixth day	0	0	0	0	0	0
Total	0	19	2	0	30	2

In cases 2 and 3 the patients were not given the usual test treatment as there was not sufficient time before the hookworm survey in New Guinea was closed, but hookworm ova had been found in the stools of each. The three men continued in good health during several weeks of observation after these tests.

CONCLUSIONS

1. Oil of chenopodium, injected intramuscularly, has little vermicidal action on hookworms, but in 1 case apparently had a marked action on whipworms.

2. Oil of chenopodium, when injected intravenously, in doses of 1.5 to 2 cc. in 3 cases, apparently had a marked vermicidal action on Trichuris; little action on Ascaris; and none on hookworms.

3. This finding suggests that the intravenous injection of suitable anthelmintics may prove an effective method of removing these worms. In view of its crude form, the intravenous use of oil of chenopodium is not recommended as a routine treatment, but this method may be practicable with more refined products. The percentage of these worms removed can not be be stated, as the total number harbored by the test cases is not known. However, an idea of the usual number of Trichuris harbored by New Guinea natives may be gained from the postmortem records of 32 inhabitants from various parts of the territory which showed an average of 4.2 of these worms per person.

The possible efficacy of anthelmintics administered intramuscularly or intravenously on whipworms deserves experimental investigation. The possible effects on the patients of this method of treatment are such that this procedure appears to be contraindicated for routine treatment of a condition like whipworm infestation which is usually not a serious matter. However, an examination of the feces for worms in the case of persons under intravenous treatment for other diseases with tartar emetic, mercury, novar-senobenzol, emetin, quinine and other products, might furnish clinical information of value—and experiments on animals, such as dogs, might furnish definite experimental information.

CONCLUSIONES

1. El aceite de quenopodio, inyectado intramuscularmente' tiene poca acción vermífuga sobre los uncinarias, pero en uno de estos casos ejerció acción marcada en contra de los tricocéfalos.

2. En tres casos en que fue inyectado el aceite de quenopodio, intravenosamente, en dosis de 1.5 a 2 cc., hubo acción vermífuga marcada sobre los tricocéfalos; muy poco efecto sobre los ascárides; ninguna acción en contra de los uncinarias.

3. Estas observaciones sugieren que la inyección intravenosa de antelmínticos apropiados puede resultar en un método efectivo para atacar estas lombrices. Debido a su forma cruda este aceite no puede recomendarse como tratamiento de rutina pero el método podría resultar de utilidad práctica con productos refinados. No se puede expresar el porciento de esta clase de lombrices expelidas pues se desconoce el total de ellos alojados por los individuos observados. Sin embargo, se puede obtener una idea aproximada del numero corriente de tricocéfalos presentes en los nativos de la Nueva Guinea, examinando el contaje post-mortem de 32 habitantes de varias partes del territorio. Estos demuestran que existe un promedio de 4.2 guzanos por persona.

4. La posible eficacia de la administración intramuscular o intravenosa de antelmínticos en contra de los tricocéfalos, merece investigacion experimental. Los efectos sobre el paciente hacen que el tratamiento este contraindicado como medida de rutina ademas de tratarse de una condición que, como esta, no es asunto de mucha seriedad. Sinembargo, el exámen de la excreta de individuos bajo tratamiento para otras enfermedades con tártaro emético, mercurio, novarsenobenzol, emetina, quinina y otros productos, podría suministrar información clínica de valor. Asi mismo los experimentos con ciertos animales como el perro.

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KALA AZAR IN THE SUDAN WITH SPECIAL REFERENCE TO ITS TREATMENT BY TARTAR EMETIC

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INTRODUCTION

Since Neave (1) and Cummins (2) first reported the existence of kala azar in the Sudan, investigations for elucidating its various problems have been carried out by different workers. Unfortunately, the advent of the great war prevented a continuity of research, and for several reasons, it has not been possible to continue the investigations, more especially those dealing with the transmission of the disease.

The work in the Sudan has been considerably handicapped owing to the paucity and inaccessibility of available material, for kala azar is by no means the common entity as in certain parts of India and the Far East; moreover, most of the endemic areas of the disease are somewhat difficult of access, and consequently lack the amenities necessary for research work. Notwithstanding these difficulties, many facts connected with the disease have been established, and this would appear a suitable opportunity for marshalling them, as well as recording any that have recently come to light.

DISTRIBUTION IN THE SUDAN

The first case was reported in 1904 in a boy, who apparently contracted the infection at Meshra El Rek in the Bahr El Ghazal Province; since then, no other cases have been reported from that province. Subsequent observations by Cummins (2) Bousfield (3), Thomson and Marshall (4) have shown that definite endemic areas for the disease exist in the districts bordering the Blue Nile and its tributaries, as well as in the districts adjacent to the Eritrean and Abyssinian frontiers. Two years ago, another infected area was found at Jongles Post situated in the District of Melut, in a strip of country between the White Nile and the Abyssinian frontier. Two cases have been recorded from Kordofan, and last year, one case occurred in a village situated on the Blue Nile within the precincts of Khartoum. So far, no cases have been reported from the arid districts of the Sudan.

EPIDEMIOLOGY AND ENDEMIOLOGY

No epidemics of any consequence have occurred in the Sudan. In 1913 a few cases occurred in a Company of Sudanese Troops stationed at Kurmok on the Abyssinian frontier, and in 1921 some 12 or 15 cases occurred among Sudanese Troops stationed at Jongles Post in the Melut district. The latter outbreak provided interesting data, inasmuch as it occurred at a new post established in a sparsely populated country. The troops lived in grass huts, and among the adjacent villages no cases were detected, nor did any of the few British officers, living under precisely similar conditions, contract the disease.

Kala azar, as seen in the Sudan, is usually of the sporadic type occurring in endemic areas. In these areas it has been the exception to find more than a single case of the disease in the same household or family. There is an ambulant type of the disease in which the course of the malady is comparatively mild; it is rare, and in the writer's experience, is confined to nomad Abyssinians found in the Sudan. Close association between the sick and the healthy, in the opinion of the writer, plays little or no part in the epidemiology of Sudan kala azar. In the outbreak at Jongles Post 6 cases occurred among individuals who partook of food in the same grass hut; this hut, however, was not occupied for sleeping purposes.

The part played by human carriers of the virus in disseminating infection has yet to be established.

Canine kala azar is unknown; moreover, experimental evidence has shown that the human virus can only be transmitted to dogs with the greatest difficulty.

ETIOLOGY

Age. The disease is commonly met with in late childhood and early adult life. In one instance it was seen in a male about fifty years of age. So far, it has not been recorded in young infants.

Sex. It is commoner in males; indeed, it is exceptional to find females suffering from the disease. The case recorded from Talodi in Southern Kordofan occurred in a female.

Race. No race or creed appears to be exempt from the disease; its incidence is slight among Europeans in the Sudan, but allowance should be made for the disproportion in numbers between them and the native population.

Occupation. It is commoner among the poorer, ill-nourished classes more especially among those without fixed abode, and addicted to wandering from village to village. In the province of Sennar on the Blue Nile, the disease appeared to be more prevalent among those employed in cutting timber in the wood forests.

Habitat. Cases have usually been found in villages situated adjacent to khors (watercourses) and streams; in places, moreover, where malaria is also endemic. As already stated, it does not occur in the arid desert areas of the Sudan.

Climate and seasonal incidence. The evidence collected shows that the endemic areas of the disease are more or less confined to districts where the annual rainfall is between 15 to 39 inches (400 to 1000 mm.). It is impossible unfortunately to obtain any records showing the humidity of these districts; it would appear, however, that humidity is a factor intimately concerned with the incidence of the disease. In the single instance quoted, where a case was discovered in the precincts of Khartoum, there had been an unusually high humidity associated with an unprecedented rainfall that year. Even in the endemic areas, the incidence of the disease varies considerably from year to year, and from the writer's observations, it seems to vary in direct ratio to the rainfall. There appears to be a greater incidence of the disease in the period following the rains, i.e., between August and December.

The causal organism. The Leishman-Donovan parasite, as found in the Sudan, in no way differs morphologically and culturally from the parasite causing visceral leishmaniasis in India and other countries. It can be readily cultured in Novy-MacNeal-Nicolle's medium at a temperature of 22°C.

Animal experiments. The Sudan virus can be easily transmitted to the grey monkey (*C. sebaeus*), red monkey (*C. rubra*), and to the jerboa (*J. gordoni*), and gerbil (*G. pygargus*). Dogs can only be infected with difficulty, massive doses of the virus being necessary to produce an infection even in young pups. Rabbits, guinea-pigs, cats, kittens, white mice, white rats, and pigeons have so far proved refractory. Local lesions, followed by a general infection, have been produced in monkeys by vaccination as well as by subcutaneous inoculation with emulsions of infected spleen, liver, and bone marrow. The grey monkey has also been successfully infected by means of feeding experiments (5).

The results obtained by experimental inoculation of animals indicate a close similarity between the Sudan and the Indian virus of kala azar.

METHOD OF TRANSMISSION

There is little likelihood of this problem being solved in the Sudan until laboratories are established in the endemic areas of the disease, where an intensive study of all the possible factors concerned could be carried out. In the writer's opinion two essential points connected with this problem await solution (*a*) the part played by man as a reservoir of the virus and so responsible for maintaining or spreading infection in endemic areas (*b*) the part played by insects, bloodsucking or otherwise, as vectors of the virus. If man acts as a reservoir of the virus, there are only two possible routes by which the parasites can escape from the human tissues, viz., from the peripheral blood through the agency of some blood-sucking insect, or from the feces.

What evidence exists to show that the parasite occurs in the peripheral blood? From a large amount of material examined in the Sudan, the evidence is mainly in the negative, but it should be borne in mind that the material submitted for examination has been taken from cases in hospital, and few attempts have been made to prove its presence by cultivation experiments with peripheral blood. Marshall (4) working in one of the endemic areas found leishmania parasites in the peripheral blood of 86 per cent of cases. The writer, however, has not been so successful in cases admitted to hospital in Khartoum. It may be that stress is a determining factor as regards the flooding of the blood stream with parasites. Stress plays an important part as an exciting factor in latent malaria producing not only attacks of fever but also parasites in the peripheral blood; it may well do so in kala azar cases exposed to the effects of sun, fatigue, and insufficient food. Epidemiological evidence in the Sudan shows that it is the exception to find more than one individual infected in the same household; this evidence, however, would not rule out the theory of the disease being insect borne, in view of the fact that it is the exception to find parasites in the peripheral blood of cases suffering from kala azar; moreover, it may be necessary for blood-sucking insects infected with the virus to survive a certain period before being capable of transmitting infection, as is seen in the case of the tsetse fly and human trypanosomiasis.

In India (6) striking evidence has been produced to show that kala azar is a house or site infection; no such evidence exists in the Sudan.

ENTOMOLOGICAL SURVEY OF ENDEMIC AREAS

Some years ago, the writer made attempts to obtain a collection of blood-sucking diptera and insects in the endemic areas situated in the Blue Nile districts. A tour of these districts was carried out two months after the rainy season and the following obtained:

Mosquitoes: *P. costalis*
Sandflies: *P. minutus, P. pappataci*
Bugs: *C. lectularius L. militaris*
Ticks: *A. persicus, A. brumpti*
Lice: *P. capitis, P. vestimentarum*
Flies: *M. domestica*
Fleas: *C. canis, X. cheopis*
Cockroaches: *P. orientalis*

Most of these were obtained (*a*) by placing flypapers on the walls of huts occupied by cases of kala azar, (*b*) from native beds used by kala azar cases, and from dogs, fowls, and rats in close association with the sick. A certain number were set aside for dissection and microscopic examination with the object of finding evidence of Leishmania parasites in the gut or salivary glands; others were dissected and emulsions inoculated into susceptible animals. The results obtained were negative.

The possibility of direct fecal transmission of the disease from infected to healthy subjects has been considered, but no facts to substantiate such a theory have been produced. The feces of kala azar cases with dysenteric symptoms have been repeatedly examined, and also cultured, with negative results.

Feeding experiments with feces have also failed to produce the disease in monkeys. No evidence has so far been forthcoming to indicate that a helminthic agency is concerned in disseminating the parasites.

The fact that the disease is frequently ushered in with dysenteric symptoms suggested the possibility that infection may occur via the alimentary tract. In the case of animals this was proved by the writer (7) some years ago and subsequently confirmed by other observers; in the light of Marrian Perry's (8) recent observations on the occurrence of leishmania parasites in the intestinal tissues of a case of Indian kala azar, this possible route of infection deserves more consideration.

In what manner the kala azar virus is acquired by man still remains a mystery. The evidence in the Sudan tends to show that man is the intermediate host in which an asexual life of the parasite can take place. If this be so, then it is probable that the part played by man as a reservoir of infection is practically negligible. Some definite host—a blood-sucking insect or one ingested by man—in which the Leishmania parasite occurs and develops as a natural infection, is probably concerned in the transmission of the disease.

The researches of Patton (9) and Cornwall (10) indicate that some blood-sucking insect is implicated in the transmission of the virus of Indian kala azar; as regards the Sudan, such a hypothesis remains "not proved."

There appears to be no relationship between the incidence of cutaneous leishmaniasis (*L. tropica*) and kala azar. In the endemic areas of kala azar, cutaneous leishmaniasis is rarely seen, whereas in the more northern arid areas in which kala azar does not occur, cutaneous leishmaniasis of recent years has shown an increasing prevalence; moreover, animal experiments have shown that the virus of *L. tropica* is quite distinct from that of kala azar.

It is of interest to note that circumstantial evidence incriminating Simulidae and Phlebotomus flies as possible carriers of *L. tropica* is very strong.

Several workers have laid stress on the relationship between canine and Mediterranean kala azar; in India and the Sudan canine kala azar is unknown: moreover experimental evidence has shown that the dog can only be infected by using massive doses of the virus.

PATHOLOGY

This need not be described in detail as the pathology of Sudan kala azar is very similar to that of Indian and Mediterranean kala azar; the most marked changes occur in the spleen, bone marrow, liver and large intestine.

Blood. A leucopenia with a diminution in the number of red cells, and a fall in the hemoglobin percentage represents the chief changes in the blood, while the differential leucocyte count shows an increase in large mononuclears and large lymphocytes, at the expense of the polymorphonuclears and eosinophiles. The marked diminution, or even absence, of eosinophiles is a constant feature of the disease.

The eosinophile ratio is also intimately concerned with the prognosis, an increase of eosinophiles being usually associated with an improvement in the patient's condition.

SYMPTOMATOLOGY

The clinical manifestations require little comment as they resemble in the main those described in India and elsewhere. In the Sudan, the acute and sub-acute types are more commonly seen; the more chronic form of the disease is only occasionally met with. From data obtained, the incubation period in the Sudan averages about two months; it would appear to depend on the physical condition of the patient at the time of infection.

Among the Sudan cases observed by the writer, profound asthenia appears to be the most striking symptom in advanced stages of the disease.

COMPLICATIONS

Diarrhea, dysentery, and epistaxis are the commonest. *Cancrum oris* and gangrene of the lung occasionally occur and invariably are precursors to a fatal termination.

Of the diseases associated with kala azar in the Sudan, malaria, ankylostomiasis and filariasis have been noted. As regards the first mentioned it should be stated that neither malarial schizonts nor gametes have been found in the large amount of kala azar material submitted for examination. The presence of malarial pigment has been the only indication of a malarial infection, probably acquired prior to the infection with kala azar.

IMMUNITY

Neither race, age nor sex influence the question of natural immunity against the disease. In the rare more chronic forms of the malady, the patient may be capable of acquiring a certain degree of immunity, and in the end may recover, but such cases must be considered exceptional. Cases of recovery followed by reinfection have not been seen by the writer.

DIAGNOSIS

This is dependent on the finding of the parasites in the blood or tissues of the infected individual. In the very early stages of the disease, diagnosis is by no means easy, and laboratory aid is usually necessary.

From a clinical point of view, cases of fever unchecked by adequate quinine administration, associated with anemia and enlargement of the spleen, occurring in endemic areas of kala azar, should be regarded with suspicion. In early cases temperature charts will invariably show intervals of apyrexia; as the disease progresses, four hourly temperature charts will usually indicate a double or treble remission daily. The pulse rate even in the afebrile periods is accelerated and is an important sign in assisting the diagnosis. In the Sudan, examination of the peripheral blood rarely reveals the causal parasites; if a leucopenia is noted together with an increase of large mononuclears and large lymphocytes and a diminution or absence of eosinophiles, it is justifiable to establish definitely the diagnosis either by the examination of liver or spleen puncture smears. The procedure of peripheral blood culture has little to recommend it in view of the uncertainty of the result, and of the impossibility of carrying it out in stations where laboratory aid is lacking; delay in diagnosis certainly affects the prognosis.

Liver puncture should be tried in cases where there is any contraindication to spleen puncture, the object being to obtain a little of the liver substance for smear preparations. Cultures of the liver blood may also be attempted.

Splenic puncture. If this be contemplated, it is advisable to raise the coagulability of the patient's blood by the administration of 15 grains (1 gram) *t.i.d* of calcium lactate for a day or two prior to the operation. The operation is carried out with aseptic precautions, a small, dry, sharp hypodermic needle and syringe being employed. The patient is requested to avoid unnecessary movements while the needle is inserted into the most prominent part of the spleen. Sufficient splenic material is withdrawn for spreading two films. After the operation a firm binder is applied to the abdomen of the patient and strict rest for twenty-four hours ensured.

Contraindications for splenic puncture. If the above technique be employed, the danger from hemorrhage is considerably minimized. *The operation, however, should never be undertaken before an examination of the peripheral blood films has been carried out.* It is contraindicated (*a*) if there be evidence of leukemia, (*b*) in severe bronchitis, (*c*) if there be marked abdominal distension due to ascites, (*d*) if the coagulation time of the patient's blood exceeds five minutes.

Other methods for diagnosis have been recommended, e.g., excision of a lymphatic gland, skin vesication, and marrow puncture, but need only be considered if splenic puncture is contraindicated. The formol-gel test (11) has been recently advocated by workers in India as an easily applied diagnostic test for kala azar. A drop of blood serum obtained from a clot is placed on a glass slide which is inverted over a watch glass containing a few drops of commercial formalin. The formalin vapor arises and acts on the serum in a few minutes. In cases of kala azar the serum will solidify in the form of a stiff jelly.

Diagnosis from post-mortem material. As the parasites rapidly degenerate after death, the post-mortem examination should be carried out with as little delay as possible. The writer has 2 cases in mind where the parasites had disintegrated in the spleen and liver to such an extent as to be unrecognizable, but were comparatively well preserved in smears taken from the bone marrow. Examination of the bone marrow should never be omitted in the post-mortem examination of suspected cases of kala azar.

Differential diagnosis. Laboratory aid is usually necessary to differentiate the diseases in the Sudan which bear similarity to kala azar in its various stages. Of these, mention may be made of the enteric group of diseases, undulant fever, infantile splenomegaly, the leukemias, ankylostomiasis, schistosomiasis, trypanosomiasis, Bantis' disease, syphilitic splenomegaly, miliary tuberculosis of the spleen, and malignant disease affecting the abdominal viscera.

PROGNOSIS

This is invariably serious; only a very small percentage of cases terminate in a natural recovery. Cancrum oris, pneumonia, and gangrene of the lung are complications usually followed by fatal results. Some indication as regards the prognosis can be gauged from periodic examination of the blood, an increase of eosinophiles denoting a favorable prognosis.

The mortality rate of the disease has certainly been lessened since the introduction of antimony and its salts as therapeutic agents but the toxic properties of antimonial preparations necessitate considerable caution, for their administration has not always proved successful even in early stages of the disease.

TREATMENT BY INTRAVENOUS INJECTIONS OF TARTAR EMETIC

Tartar emetic has been given a fairly extensive trial in the treatment of Sudan kala azar, and the writer has had several opportunities of observing closely the results obtained by the intravenous administration of the drug in cases of kala azar occurring among adult males. Some cases, even in an advanced stage of the disease, have undoubtedly benefited and have been cured; on the other hand, fatal results have occurred among several cases which, from a clinical point of view, should not have succumbed. Observations which have been carried out on these fatal cases indicate that death resulted from overdosage with a drug empirically employed in individuals obviously intolerant to the toxic action of tartar emetic. This intolerance was shown by collapse followed by a fatal result.

It is not the purpose of this paper to record each case in detail but rather to call attention to the fact that tartar emetic—the salt of antimony usually employed in the treatment of kala azar in the Sudan—is possessed of toxic properties warranting careful administration, even in early stages of the disease, and to advocate a rational use of this specific drug in all cases of kala azar, and by so avoiding overdosage, to lessen the mortality rate.

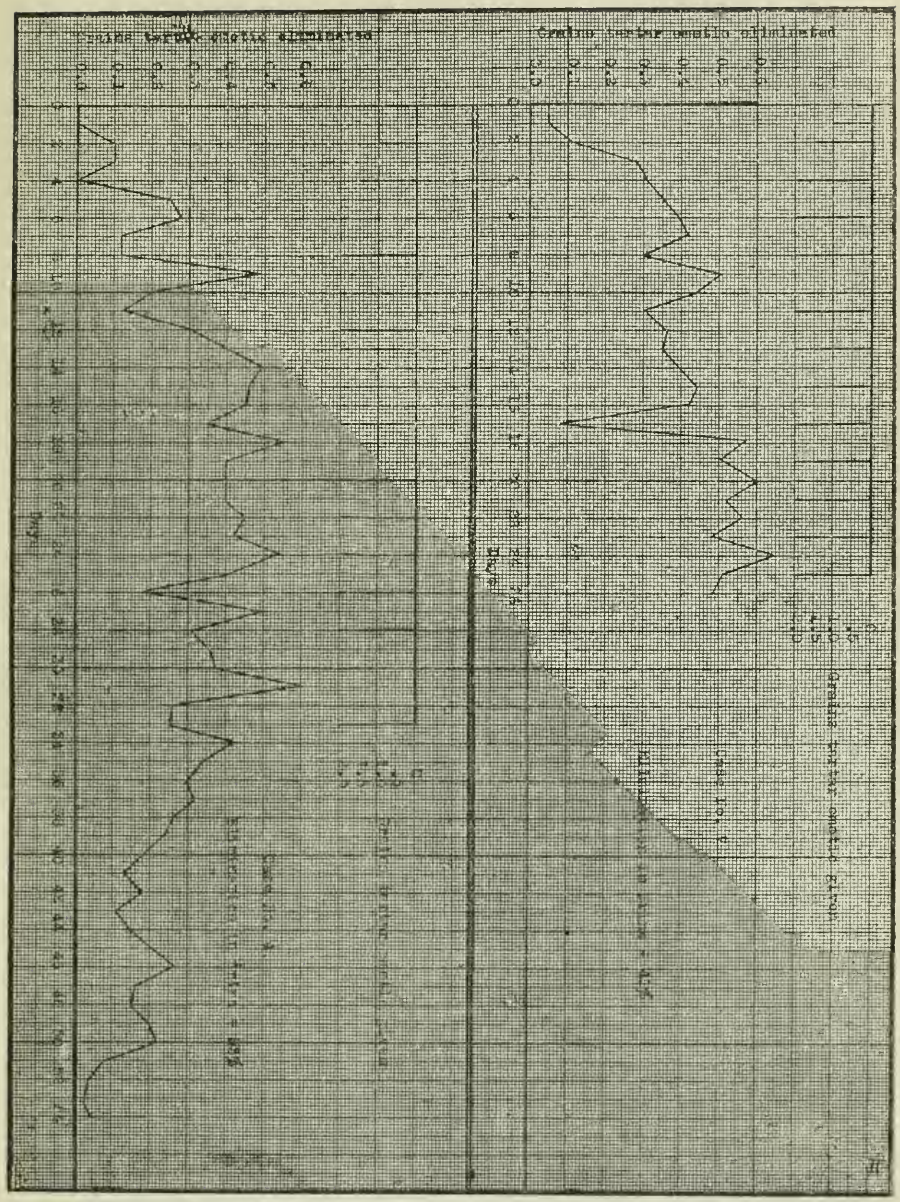
The observations recorded here are based on the results following the treatment of 15 cases with 13 deaths, as well as other fatal cases known to the writer.

Of the 13 fatal cases mentioned, 9 represented early types of the disease in which the physical condition of the patients was good; the remainder represented moderately severe types of Sudan kala azar where pyrexia had been existent for some time, and where asthenia was a noticeable feature.

All these cases were treated with intravenous injections of tartar emetic on the lines advocated by experienced observers in India. It may be stated that the drug, already prepared in ampoules suitable for intravenous injection, was obtained from a reputable firm of English chemists. The dosage consisted of 0.5 grain of tartar emetic for the initial injection followed two or three days later by the injection of an amount equivalent to 1 grain increased at two to three days interval to 1.5 grains and in occasional instances to 2 grains. If any evidence of untoward symptoms appeared such as vomiting and marked diarrhea, the further use of the drug was suspended for a time and then continued. The presence of albuminuria was always considered a contraindication to treatment by tartar emetic.

In some of the fatal cases, the result of treatment showed a decline in the temperature, and a decrease in the size of the spleen associated however with an increasing asthenia of the patient. Histopathological examination of the organs showed marked fatty degeneration of the liver, heart, and kidneys very similar to those recorded by the writer (12) in a fatal case of bilharzia treated by tartar emetic. There was little doubt that the drug by its cumulative action was responsible for the fatal results.

In one of the fatal cases, the patient had received a total of 8.5 grains of tartar emetic by intravenous injections. Sixty-seven days after the last injection the patient died. The liver, heart, and kidneys showed profound fatty changes and chemical analysis of the liver showed the presence of antimony. In another fatal case which had received a total amount of 3 grains of tartar emetic, 0.125 grain of antimony was recovered in the liver eight days after the last injection.



Graphs Illustrating the Percentage of Tartar Emetic Eliminated in the Urine of Two Cases that Died During Treatment

The accompanying protocol shows to what extent the drug was eliminated in the urine of two cases that died during treatment. In the one case 41 per cent and in the other 53 per cent of the drug was eliminated via the kidneys.

It is of interest to note that Levy and Dimmitt (13) have recently shown that tartar emetic given intravenously even in small doses over a comparatively short period is capable of producing a decided increase in the non protein nitrogen elements of the blood—a fact, which they state, should be borne in mind during the rather free use of this drug in the treatment of granulomatous conditions.

Christopherson (14) in a paper dealing with the treatment of three cases of Sudan kala azar by tartar emetic injections states "that it is useful to remember a tolerance for the drug is easily established so that the initial dose can be rapidly increased." It is difficult to reconcile this statement with the results obtained for, of the three cases recorded, two died and one recovered. To attribute fatal results to sudden cardiac dilatation is ignoring the toxic part played by tartar emetic in such cases.

Some cases show no tolerance for the drug while others, although exhibiting no symptoms of vomiting, abdominal pain, and purging, show signs of increasing asthenia and eventually succumb as the result of the deficient elimination and cumulative action of the drug.

Colonel Biggar, Principal Medical Officer, Egyptian Army, has had similar disappointing results in the use of tartar emetic on the lines advocated by Indian observers, and as a result, has been compelled to alter the method of dosage previously employed.

The dosage of tartar emetic recommended in the treatment of kala azar

As regards tartar emetic therapy, there appears to be a prevailing impression, and certainly an incorrect one, that kala azar should be treated on the same lines as Bilharziasis. The two diseases are totally different; in kala azar the profound blood changes and associated asthenia less befit the patient to withstand the toxic cumulative action of tartar emetic; moreover, the absence of vomiting, abdominal pain, and purging following its administration should not be accepted as signs of tolerance for the drug, and do not justify its exhibition in rapidly increasing doses. Increasing asthenia and loss of weight are significant indications for the more cautious use of the drug. In India, the weight of the patient is considered as a factor influencing the dosage; in the Sudan, as far as adult males are concerned, experience has shown that this factor is negligible, the capacity for eliminating the drug is a more important one.

With these points in view, it appeared rational to adopt a less intensive form of treatment, one which would be considered comparatively safe as well as curative. It was found from experience that an initial dose of 0.5 grain of tartar emetic given intravenously, followed seven days later by an injection of 1 grain, and subsequently 1 to 1.5 grains at ten to fourteen days intervals produced satisfactory results. This method has been employed by Colonel Biggar with great success even in advanced stages of the disease, and so far, no fatal results have been recorded. Loss of weight and increasing asthenia were taken as indications for lessening the quantity of the drug, and increasing the intervals between the injections. In cases complicated with dysenteric symptoms, smaller quantities of the drug should be employed; every effort should also be made to maintain the nutrition of the patient by special dietetic measures.

THE DOSAGE REQUIRED TO EFFECT A CURE

Short of cultural tests, either of blood or splenic material, it is difficult to state when a patient is cured; moreover, there is always considerable trouble in following up the after history of cases discharged from civil hospitals.

For practical purposes, however, it would appear that a disappearance of the pyrexia, and an increase in the body weight with a return of the spleen to its normal size would indicate a probable cure.

The writer has observed only 1 case of undoubted relapse which had been discharged from hospital as cured.

The amount of the drug necessary to effect a cure would depend on the intensity of the infection, or in other words, the severity of the case. Cures have been obtained after the administration of a total of 8 grains of tartar emetic. In one very early case that came under the notice of the writer, a total of 6.5 grains effected a cure; in this instance, the period of treatment extended over two months, a point which, in the writer's opinion, was responsible for the happy result obtained.

In a case of a moderately severe infection, that ended fatally after a total of 16 grains of tartar emetic had been administered over a period of thirty-eight days, Leishmania parasites, in a viable form, were found in fair numbers in the spleen. Splenic material—obtained two hours after the death of the patient—when cultivated in Novy-MacNeal-Nicolle media showed typical Leishmania flagellates after forty-eight hours incubation at 22°C.

Knowles (15) from experience in India, states that a 2 gram (30 grains) course of tartar emetic distributed over a period of three months is sufficient to sterilize an adult of Leishmania parasites. In the Sudan a total probably of 25 to 30 grains will effect a cure, but this amount, for reasons already given, should be distributed over a longer period than three months; by doing so, unfortunate fatalities will be avoided, and as shown by recent experience, a larger number of cures will be effected.

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RESÚMEN

El autor reseña detalladamente las investigaciones previas y la distribución del Kala Azar en el Sudán. Al tratar de la epidemiología y endemiología de la enfermedad en aquel terri-

torio, indica que no se sabe de ninguna epidemia de consecuencia que haya ocurrido. El mayor brote conocido (1921) afectó a un total de 15 soldados de las tropas sudanesas del distrito del Melut. El tipo de la enfermedad es esporádico y parece que la asociación entre enfermos y sanos no toca ningún papel en la epidemiología. El parásito causal es el de Leishman-Donovan y no difiere ni morfológicamente ni en cultivo de la leishmaniosis visceral de India y otros países. Se obtiene fácilmente en el medio de cultivo de Novy-MacNeal-Nicolle a temperatura de 22°C. Los resultados obtenidos por experimentos de inoculación animal son similares a los del virus de India. Opina que existen dos puntos esenciales en espera de solución, (1) la parte que juega el hombre como albergue del virus y por lo tanto como diseminador a las areas endémicas y (2) el papel que juegan los insectos, ya sean chupantes o no, como trasmisores. La manera precisa como se contrae el Kala Azar es un misterio. Hace un interesante manifiesto de la diagnosis y llama la atención, desde un punto de vista clínico, a aquellos casos de fiebre que no son alterados por la quinina y que estan asociados al mismo tiempo con anemia y ensanchamiento del bazo, los cuales deben ser considerados sospechosos. En las primeras fases de la enfermedad se demuestran intervalos de apirexia con invariabilidad. Durante el progreso de la enfermedad se registran remisiones dobles o triples al día. El pulso presenta aceleración constante, un punto este de mucha importancia diagnóstica. La sangre periférica no revela los parásitos. Si existe leucopenia con aumento de los mononucleares y linfocitos mayores, y ausencia o disminución de eosinófilos, es justificable el establecer la diagnosis definitiva por punción del hígado o del bazo. Tocante al tratamiento dice que el tártaro emetico, aplicado intravenosamente, ha beneficiado y curado varios casos, pero que así mismo varios otros han terminado fatalmente como resultado de dosis excesivas a individuos que parecen poseer intolerancia hacia la acción tóxica de la droga. Estas observaciones tienen por fundamento los resultados obtenidos en el tratamiento de quince casos de los cuales trece murieron. De las fatalidades, nueve recibieron tratamiento al empezar la enfermedad cuando las fuerzas físicas estaban en vigor; los restantes eran casos severos pero moderados con pirexia ya existente por varios dias. Recomienda una dósis inicial de $\frac{1}{2}$ grano de tártaro emético seguida, siete días mas tarde, por 1 grano y, subsiguientemente, por $1\frac{1}{2}$ granos a intervalos de 10 a 14 días. Este método de tratamiento ha sido tambien usado por el Coronel Biggar, Director Médico del Ejército Egipcio, con resltados lisonjeros. Tremina manifestando que en el Sudán un total de 25 a 30 granos de la droga producen cura, pero que esta cantidad, por razones ya expuestas, debe distribuirse dentro de un período de por lo menos tres meses.

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SEROLOGICAL REACTIONS IN YELLOW FEVER

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Since Noguchi's (1) reports on the transmission of yellow fever to animals and the isolation of *Leptospira icteroides* from patients and from experimentally infected guinea pigs, investigations of this organism have multiplied, and its specific rôle in yellow fever is unquestionable. Various observers have accomplished experimental transmission of the disease and isolation and cultivation of the microorganism (2), and recently the identity of the human and experimental lesions has been established in an indisputable manner (3).

The immunity reactions were carefully studied by Noguchi in connection with the demonstration of the etiological rôle of the parasite. Noguchi's results suggested the desirability of studying these reactions with a view to their practical application in early diagnosis of yellow fever, and the opportunity to carry out such an investigation presented itself during an epidemic of yellow fever in Vera Cruz. An extensive study of the serum reactions in every case was not possible, but the results obtained in a moderately large series of cases are significant and may prove useful in future investigations.

AGGLUTINATION REACTION

Of the serum reactions, the most easily accessible to the clinician because of simplicity of technique is without doubt that of agglutination; moreover, this type of reaction appears earlier and persists longer than any other. For the study of agglutination it was necessary to obtain homogeneous cultures in which the organisms are dispersed throughout the medium and yet are present in sufficient abundance. The presence of agar in the medium frequently causes spontaneous agglutination of the leptospiras and interferes with observations, hence the concentration of agar had to be reduced. The organism were found to grow well in the rabbit serum Ringer medium adopted by Martin and Pettit (4) for *Leptospira icterohaemorrhagiae.* Cultures seven to eight days old are sufficiently rich, and the free leptospiras are actively motile.

TECHNIQUE

As in the case of the Widal reaction in typhoid fever, the serum in question is diluted and mixed with the culture. The standard dilution used was 1: 100, although control tests showed that sera from persons who had not had an attack of yellow fever, or had not been vaccinated against it, did not agglutinate the organisms even in dilutions as concentrated as 1 : 20, 1 : 30, 1 : 40, while sera from persons who had had yellow fever agglutinated in higher dilutions (1: 1,000 in one case on the seventeenth day of disease). One drop of the suspected serum was mixed with 49 drops of isotonic salt solution; the serum thus diluted was mixed in equal parts with the culture. Three controls accompanied each test, one with a known positive serum, one with a known negative, and another with culture alone. Examinations were made by means of the darkfield microscope, which is essential in this technique.

In the case of the strongest reactions there was observed after a short time—ten to fifteen minutes—the formation of clumps of leptospiras, united sometimes in a curious stellar grouping in which one end of each individual was free and had a slight undulatory movement. More frequently there were voluminous masses, spheroidal and granular, from which emerged individuals which were rapidly undergoing alteration, losing little by little their activity and refringency and finally breaking up into minute granules. In other words, agglutination is accompanied by lysis. When lysis takes place the reaction is easily recognized; it differs decidedly from spontaneous agglutination of the organisms, in which the leptospiras remain structurally perfect and actively motile.

In the less intense reactions, the lytic phenomena are absent, and agglutination and immobilization only occur, but even in these instances immobilization is sufficiently characteristic to classify the reaction as positive. In general the reaction reaches its maximum in half an hour. The controls show motile organisms even after several hours, providing care is taken to avoid drying and to maintain suitable temperature conditions. The organism is most active within the range of temperature from 20° to 37°C.

The sera of healthy persons or persons suffering from diseases other than yellow fever were utilized as controls, and no positive reactions occurred among them. The sera of persons vaccinated against yellow fever (5)(6) brought about agglutination of the leptospiras, the reaction presenting the same characteristics as in the case of sera from yellow fever patients.

The action of serum taken during the early days of the disease, as may be seen from the tables, consisted in agglutination phenomena only; as time went on the action of the serum became more and more energetic and the doubtful reactions fewer; and interpretation, difficult at first, is simple by the end of the first or the beginning of the second week of illness and remains so for some time.

Among the samples of sera taken during the first three days of illness, many served the purpose of transmission of the disease to guinea-pigs, with positive results in approximately a third of the cases. Darkfield examination of every specimen taken was systematically carried out without our once encountering the parasite in human blood. In the blood of the infected guinea-pig, however, taken during the first three or four days of the disease, demonstration of the leptospira was frequently successful. Some of the samples of blood also served the purpose of direct cultivation of the leptospira.

Human and guinea-pig serum had an analogous action on the cultures, whether the latter were obtained directly from man or after passage through the guinea-pig.

Of 5 sera from patients on the first day of illness, none gave a positive reaction. Of 9 taken on the second day, 5 gave negative reactions and 4 slight agglutination which we considered doubtful. Of 12 sera taken during the third day, 5 gave slight agglutination; in 1 case the reaction was more intense. Fourteen sera were taken during the fourth and fifth day; with these there were 10 doubtful results and 1 frankly positive reaction, the latter in the case of a fifth day serum. Of 28 sera taken during the sixth day, only 2 gave negative reactions; 6 were doubtful, but in the other instances the reaction was easily appreciable and was always accompanied by lysis. One hundred and fifteen sera were taken from the seventh to twenty-seventh days, of which 14 gave doubtful and only 6 negative reactions. The diagnosis in three of the latter cases was doubtful; in the other three the disease was unquestionably yellow fever. The remaining 95 sera gave unmistakably positive reactions.

Seventeen sera from cases of jaundice of various origin were examined (infectious icterus, malaria, typhoid, arsenic poisoning, etc.). None of these gave a positive reaction.

The sera of healthy persons who had not been vaccinated against yellow fever always gave negative reactions. Some of these persons were later vaccinated, and the reaction shifted to positive. Among 11 sera of vaccinated persons, 7 positive reactions were observed with sera taken between the tenth and fifteenth day after the second injection of the vaccine.

It is not possible at present to say when the agglutinins are lost from the serum. In the sera of some immune persons who had had an attack of yellow fever several years previously agglutinins were found to exist, but many others gave a negative reaction.

To summarize, then, the reaction of agglutination is clearly appreciable from the sixth or seventh day and persists for a long time. In general it is accompanied by immobilization, disintegration, and lysis of the leptospiras.

THE PFEIFFER REACTION

The technique of the Pfeiffer phenomenon is rather more delicate than that of agglutination and requires more material. Scarcity of animals did not permit the making of Pfeiffer tests in all cases which came under observation. Moreover, many of the guinea-pigs died from secondary infection after having been punctured several times.

The culture of *Leptospira icteroides* used in all of these investigations was the one obtained by me in Vera Cruz (2). Passage of the organism through the guinea-pig had raised its virulence until it almost always infected fatally, the incubation period often being as short as two days. It was not possible to determine the minimum lethal dose for the guinea-pig, but the results in the control animals showed that it was not necessary to use as much as a drop from a capillary pipette to bring about a fatal infection.

TABLE 1

Agglutination reaction

DAY OF ILLNESS ON WHICH SERA WERE TAKEN	POSITIVE	NEGATIVE	DOUBTFUL	TOTAL
First	0	5	0	5
Second and third	1	12	8	21
Fourth and fifth	1	3	10	14
Sixth	20	2	6	28
Seventh to twenty-seventh	95	6	14	115
Total	117	28	38	183
Sera from other diseases	0	17	0	17
Normal sera	0	23	0	23
Total	0	40	0	40
Sera from persons vaccinated against yellow fever	7	3	1	11

In all cases 0.25 cc. of the serum of the patient was mixed with 2 cc. of culture of *Leptospira icteroides* and injected into the peritoneal cavity of 2 guinea-pigs, another guinea-pig being injected with culture alone as a control. The sera from patients with malaria, typhoid fever, etc., which were used as controls, always gave negative reactions. The peritoneal fluid was withdrawn for examination under the darkfield microscope every half hour for two to three hours, and at the end of one and one-half to two hours no motile organisms were encountered. The inoculations and the withdrawal of peritoneal fluid were made with a capillary pipette.

The reaction was positive in the 17 sera taken on the twelfth day or later, and negative in 1 serum taken on the eighth day. The serum does not appear to develop protective properties until convalescence.

TABLE 2

Pfeiffer reaction

DAY OF DISEASE ON WHICH SERUM WAS TAKEN	TOTAL NUMBER OF SERA	POSITIVE	NEGATIVE	REMARKS*
Eighth	1	0	1	Both animals died of typical infection
Twelfth	3	3	0	2 animals died of secondary infection, 4 on 6th to 8th day of typical infection
Fifteenth to sixteenth	2	2	0	2 animals died of secondary infection; 2 had typical infection, died 8th day
Twenty-first to twenty-seventh	11	11	0	7 animals died of secondary infection; 15 passed through a typical infection, of these 2 died, 8 recovered, 5 had no fever
Two months after yellow fever	1	1	0	1 animal died of secondary infection; 1 recovered

* All controls died; infection typical.

L. M., fifteenth day of illness. At the first examination, one-half hour after inoculation, very few motile leptospiras were found; almost all had been agglutinated and were becoming granular. On the second examination no leptospiras were found. The guinea pig died two days later of secondary infection. The second guinea-pig passed through a typical *icteroides* infection and died after eight days. The control animal died after six days, having had a typical infection. In both the period of incubation was two days.

R.C., sixteenth day of disease. Results identical with the preceding. The first guinea-pig, punctured three times, died of intercurrent infection three days later. The second guinea-pig, and the control, passed through a typical yellow fever infection and died in eight and seven days respectively.

Av. P., yellow fever two months previously. The control died on the fifth day, after a typical infection. The first guinea-pig, in which the results of the test were the same as in the two preceding cases, died of secondary infection the following day. The second, after three days of incubation, showed an elevation of temperature which persisted for four days. Slight icterus appeared on the third day of fever. The animal recovered. *Leptospira icteroides* was recovered from blood taken from the animal on the third day of fever; the culture obtained induced fatal infection. The serum taken twenty days later gave a positive Pfeiffer test in a normal guinea-pig; the latter remained well.

B. L., eighth day. Reaction negative. Both guinea-pigs survived puncture but died six days later; typical infection. The control died on the same day.

Three sera taken on the twelfth day were tested. Reaction positive. Two guinea-pigs died of intercurrent infection the following day. The four others and the controls passed through typical infection, dying between the sixth and eighth days.

Eleven sera were taken between the twenty-first and twenty-seventh days. All gave a positive reaction. Seven guinea-pigs died of intercurrent infection; 5 had no temperature reaction, and the sera of three of these conferred immunity; 8 presented typical yellow fever and died on the fifth to sixth days. All the controls died on the fifth to eighth days.

These results are summarized in Table 2.

COMPLEMENT FIXATION REACTION

Our experiments on complement fixation were few and the results unsatisfactory. The antigen used was that recommended by Noguchi (7). The cultures in fluid medium were killed by heating at 55°C. and were titrated carefully for anticomplementary property. Titration offered many difficulties and had to be repeated many times. The anticomplementary power of the different antigens was very variable. The quantity of antigen necessary to fix all the complement, in the presence of yellow fever serum, was in all our cases so great that it was almost an anticomplementary unit, hence the margin between the antigenic and anticomplementary units was so small as to be a serious source of error. The alcoholic extract of the liver of the infected guinea pig, which we tried to employ as antigen, gave no better results. Syphilitic antigens gave intense fixation in some cases in which we had not suspected syphilis. In many cases in which the diagnosis of yellow fever was certain the reaction was negative; one serum from a malaria patient gave a more intense reaction than any other; it could not be determined whether or not the patient had previously had an attack of yellow fever.

All the sera were taken during convalescence. In all cases antisheep amboceptor prepared in the rabbit and sheep erythrocytes were used.

The results are recorded in Table 3.

TABLE 3

Complement fixation reaction

	POSITIVE	WEAK	NEGATIVE	TOTAL
Sera from yellow fever patients..........	3	9	4	16
Sera from other diseases..................	1	4	5	10
Normal sera..............................	0	2	3	5

CONCLUSIONS

Serum from 183 cases of yellow fever, 17 cases of other diseases, and 23 normal individuals were studied for the purpose of determining the existence of specific agglutinins for *Leptospira icteroides*. The results showed that the serum of persons attacked by yellow fever acquires agglutinating properties for the leptospira which are easily demonstrated by the beginning of the second week of the disease. Sera from persons who had had yellow fever years previously usually gave negative reactions. Normal sera or sera from diseases other than yellow fever do not contain agglutinins for *Leptospira icteroides.* On the other hand, sera from recently vaccinated individuals usually gives positive agglutination. The facility of the technique and the precision of the procedure makes it the choice for diagnosis.

Agglutination by the sera is differentiated from spontaneous agglutination in being always accompanied by immobilization and frequently by lytic phenomena. The lysins probably appear in the serum at the same time as the agglutinins.

Pfeiffer's reaction was constantly present in the sera from 17 persons convalescent from yellow fever. It was negative in the serum from a case on the eighth day of illness.

The immunizing property of the serum, as evidenced by a positive Pfeiffer reaction against *Leptospira icteroides,* makes its appearance late, perhaps after the second week, being especially easy to demonstrate during convalescence. The Pfeiffer reaction is therefore of less practical applicability in diagnosis than agglutination, but it constitutes valuable confirmatory evidence of yellow fever.

In our experience, complement fixation is subject to numerous sources of error, which deprive this reaction of all practical usefulness.

CONCLUSIONES

Se estudiaron 183 casos de fiebre amarilla, 17 de otras enfermedades y 23 individuos normales, con el propósito de determinar la existencia de aglutininas específicas para la *Leptospira icteroides.* Los resultados demuestran que el suero de personas atacadas de fiebre amarilla adquiere propiedades aglutinantes para la leptospira, propriedades que son fácilmente puestas en evidencia a partir del principio de la segunda semana de la enfermedad. El suero de personas que en años previos tuvieron fiebre amarilla, dá resultados negativos generalmente. Sueros normales o sueros de otras enfermedades que no sean fiebre amarilla, no contienen aglutininas para la *Leptospira icteroides.* Sin embargo, los sueros de individuos recientemente vacunados, usualmente dan aglutinación positiva. La facilidad de la técnica y la precisión del prodecimiento lo hacen el de elección para el diagnóstico.

La aglutinación por el suero se diferencia de la espontanea, en que siempres e acompaña de inmovilización y con frecuencia de fenómenos líticos. Las lisinas aparecen en el suero probablemente al mismo tiempo que las aglutininas.

La reacción de Pfeiffer fué observada con constancia en los sueros de 17 personas convalescientes de fiebre amarilla. Hubo reacción negativa en el suero en uno de los casos al octavo dia de la enfermedad.

La propiedad immunizante del suero contra el *Leptospira icteroides* hace su aparición un poco mas tardíamente (acaso después del segundo septenario), siendo facil de poner en evidencia con especialidad durante la convalescencia del infermo, lo que reduce sus aplicaciones prácticas; pero es sin duda una de las pruebas de más valor para el diagnóstico retrospectivo.

A juzgar por nuestra experiencia, la prueba de fijación del complemento está sujeta a numerosas causas de error, que le quitan toda utilidad práctica.

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FLEAS OF PANAMA, THEIR HOSTS, AND THEIR IMPORTANCE

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When the Indian Plague Commission definitely established in 1905 the exact relationship of fleas in the transmission of bubonic plague from rat to rat and from rat to man, these insects at once became of great importance, from the standpoint of disease transmission, to practically all countries. This is especially so in countries having sea ports, where the danger of introduction of plague is always present to some extent, and Panama in particular being one country to come under this category.

Although some investigations have been made in regard to the fleas infesting rats in Panama, no general survey to determine the various species attacking other animal life on the Isthmus, their prevalence, the hosts selected by them and their importance in relation to plague, has yet been carried out. While the following list undoubtedly contains but a few of the species present, it is as complete as our knowledge of the fleas of Panama permit at this time and is presented as a contribution towards such a survey.

Xenopsylla cheopis Rothschild

The Indian rat flea. From a plague standpoint this species is the most important of the fleas present in Panama. It is commonly found infesting the rats and mice that usually live in more or less close association with man. These consist of the brown rat, *Mus norvegicus* Erxleben, the black rat, *Mus rattus* Linnaeus, the roof rat, *Mus alexandrinus* Geoffroy, and the house mouse, *Mus musculus* Linnaeus.

In 1909 and 1910 the late A. L. Jennings (1) conducted an investigation of the rats and fleas of the terminal cities, Panama and Colon, and the Canal Zone. This work, undertaken by the direction of Dr. S. T. Darling, formerly Chief of the Board of Health Laboratory, Ancon, C. Z., was quite probably the first systematic effort made toward classifying the rats and the fleas infesting them in Central America. During this investigation, which extended from December 10, 1909, to July 10, 1910, a total of 2394 rats and mice were examined and 3022 fleas found, averaging 1.26 fleas per rat. Of this number, 2966, or 97.9 per cent, were rat fleas, *X. cheopis,* and 56, or 2.1 per cent, were cat fleas, *Ct. felis.* On 577 of these rats that were captured in the city of Panama during the latter period of this work a total of 1365 fleas, or 2.37 per rat, were present. All were rat fleas except 28 *Ct. felis.*

Anti-rat work has been carried on in the cities of Panama and Colon, and the Canal Zone, by the Sanitary Department of the Canal Zone, for a number of years. The rat proof construction of all new buildings, rat proofing of old buildings and the demolition of wooden docks and piers at the terminal ports and replacing them with new concrete structures have not only reduced the number of rats but undoubtedly also contributed toward changing their habits to a considerable extent. With the elimination of many conditions favorable for propagation in the houses and other buildings, numbers of them have been forced to seek more out of doors breeding places which, owing to greater exposure to the atmospheric conditions, has quite probably had some effect on the flea infestation of the different species.

During the period between October, 1920, and August, 1921, the writer, while Entomologist at the Board of Health Laboratory, Ancon, C. Z., examined a number of rats and mice for the purpose of determining the species and abundance of fleas infesting them. These rats were captured in wire traps and brought alive to the laboratory where they were transferred from the traps to large, glass jars and chloroformed. The use of the glass jars as receptacles in which to kill the rats proved very satisfactory as it was quite impossible for any of the fleas to escape and any that left the host before being affected by the chloroform were easily found in the jars. All the fleas collected were rat fleas, *X. cheopis*, with the exception of one which was a cat flea, *Ct. felis*. The results of these examinations are given in the following table:

SPECIES OF RATS	PANAMA		COLON		CANAL ZONE		TOTALS		FLEAS PER RAT
	Rats	Fleas	Rats	Fleas	Rats	Fleas	Rats	Fleas	
Mus norvegicus..........	32	138	16	0	64	26	112	164	1.46
Mus rattus...............	19	153	5	3	52	76	76	232	3.05
Mus alexandrinus........	7	63	3	5	7	12	17	80	4.70
Mus musculus...........	115	7	517	14	388	8	1020	29	0.028
Totals.................	173	361	541	22	511	122	1225	505	0.41

The average of 0.41 fleas per rat as shown above for the combined total of rats and mice is a very light infestation if the mice be given the same consideration as the rats, which really should not be done. The European mouse flea, *Ctenopsyllus musculi* Duges, that is commonly found on house mice in some parts of the United States and in Europe has not been found in Panama, and the rat flea, *X. cheopis*, apparently does not accept the mouse as a favorable host and probably makes use of it more as a temporary one than as a permanent one. This is quite evident from the fact that but 29 fleas were found on 1020 mice. A total of 476 fleas was found on the 205 rats examined, equaling 2.32 fleas per rat.

Rats and mice captured at the new cement piers and docks at Cristobal were found to be free from fleas. Forty-two rats—30 brown, 9 black and 3 roof rats—and 149 mice that were taken at these docks were uninfested.

The rats from Panama City showed a high average infestation while those from Colon had a comparatively low one. The greatest number of fleas found on one rat was 54 which were collected from a small, male, brown rat captured in Panama. Those taken from 3 half-grown, black rats from Panama totaled 103 while a roof rat was found to be acting as host for 41. A black rat captured at Pedro Miguel Locks was carrying 17 fleas and a brown one taken at the same place was infested with 8. Seventeen were also taken from a brown rat captured at the Balboa Commissary. Rats as heavily infested as these would be very apt to prove a grave menace to the population of Panama in the event of plague being introduced.

Dermatophilus penetrans Linnaeus

The chigoe flea, also variously known as "jigger," "chigger," "nigua" and "sand flea." This species is very prevalent in many of the native villages throughout the interior of Panama. Sandy regions frequented by animals that act as hosts for this flea and the earth floors of native houses sometimes become infested with great numbers of this pest. While pigs are probably its favorite host it also attacks man, dogs, cats, etc., with much freedom. Owing to the burrowing habits of the female this species is an extremely annoying one to man. The unimpregnated female and the male feed without burrowing as other varieties of fleas do but as soon as the female becomes impregnated she proceeds to work her way into the skin of the host where she remains while her eggs develop and are deposited, unless removed sooner. It is quite possible that the heat and moisture of the skin have an effect on the development of the eggs which may account for this habit. The female probably also derives nourishment from the skin during this period of oviposition. In man, the feet are where the attacks by this flea are usually made. It burrows into the skin in the region of the toenails, in the creases on the underside of the toes and also in the tough cuticle on the soles of the feet. Other parts of the body, even the fingers beneath the nails, are also subject to attack but not frequently. Many of the inhabitants of the native villages do not wear shoes, and many others wear only sandals, and are therefore exposed to the pain and discomfort caused by the attacks of these parasites. In April, 1917, two American soldiers were admitted to Ancon Hospital owing to infestation with this flea. They both had been members of a mapping party encamped near Chorrera and probably became infested at the houses in the town. One of them had about 30 of these fleas imbedded in his feet when he arrived at the hospital. Five of them were located in a row in the crease under the big toe on the left foot. Due to the irritation caused by the fleas in the skin, ulcers had formed; some of which were confluent, and both feet were badly swollen and very painful. The second case was not as severe, there being a smaller number of fleas imbedded and the infestation having been of shorter duration before coming to the hospital. However, this man developed a rather severe, inguinal adenitis as a result of the infestation.

Pulex irritans Linnaeus

The human flea. Although this species is present in Panama it does not seem to be at all prevalent. The fact that none of the rats examined were infested with this flea would indicate that it is not common in the houses of Panama, for while man is the favorite host it is also occasionally found on rats in localities where it is very numerous. Only one instance of this species being present in a habitation in sufficient numbers to cause the occupants much annoyance came to the attention of the writer while on the Isthmus. This occurred at Camp Muru in May, 1921. This was a labor camp located in the jungle near the Chagras River, on the Atlantic side of the Isthmus. The habitations of this camp were of a temporary nature, being built of poles and thatched with palm leaves. During the latter part of the dry season these dwellings became badly infested with fleas, which caused the men considerable annoyance until control measures were carried out. Specimens of these fleas were received at the laboratory, through the kindness of Sanitary Inspector J. B. Shropshire, Panama Canal Department, United States Army, and upon examination were found to be *P. irritans*. As this species is so prevalent in some of the Central and South American countries as well as in certain sections of the United States, it is difficult to understand why it is not more common throughout the Isthmus and it is quite possible that the excessive moisture in the atmosphere of Panama may have an inhibitory effect on its propagation. Owing to this flea accepting both man and rats for hosts it may be considered as a possible agent in the transmission of plague.

Echidnophaga gallinaceous Westwood

The chicken flea or "sticktight." This flea seems to be present in some localities in Panama and absent in others. Chickens are the favorite hosts but other fowl, dogs, cats, rats and sometimes man are also attacked. This species gathers in masses on the head of a host, especially when attacking a young chicken, where the females attach and burrow into the skin to some extent. The irritation caused by this burrowing produces an exudate which forms about the minute lesion and almost covers the flea. It is believed that the females deposit their eggs in these small lesions, the larvae dropping to the ground soon after emerging. While this flea is probably of but little consequence from the standpoint of affecting human health it is of economic importance to the poultry owner as the irritation caused by its attacks often has a detrimental effect on young fowls.

Ctenocephalus felis Bouche

The cat flea. This species is quite prevalent, there probably being but very few dogs or cats in Panama that are not infested with it. However, these infestations are undoubtedly no heavier, or even as heavy, per animal than they are in many parts of the United States. Occasional reports of houses being infested with fleas that annoyed the inhabitants were found upon investigation to be due to the presence of this species and not to the human flea.

Ctenocephalus canis Curtis

The dog flea. Specimens of this species were found on a dog that was brought to the laboratory at Ancon from Swan Island as a rabies suspect. This was the only time the writer encountered *C. canis* on the Isthmus.

Rhopalopsyllus dunni Jordan and Rothschild

Specimens of this species were found on cotton rats, *Sigmodon hispidus chiriquensis* Allen, captured near Miraflores. These rats are very numerous in areas covered with thick grass and small bushes in the vicinity of towns in the Canal Zone and in places in the jungle that have been partly cleared of forest growth. In 1921 they were quite common at Balboa, being present in numbers in the grass near the town and on the filled area opposite the Balboa Station. Several hundred of them were killed daily in the scrub brush and grass on an area east of Miraflores Locks, by men engaged in clearing off the growth for an aeroplane landing place.

Rhopalopsyllus klagesi Rothschild

This species was found on the Panama spiny rats, *Proechimys semispinosus panamensis* Thomas, captured in the long grass near Pedro Miguel and on an ocelot, *Felis pardalis mearnsi* Allen, killed near La Cabima.

Rhopalopsyllus cacicus Jordan and Rothschild

Specimens of this flea were present on a three-toed anteater, *Tamanduas tetradactyla chiriquensis* Allen, killed at Alhajuela, on the Chagras River, and on an ocelot, *Felis pardallis mearnsi* Allen, from La Cabima.

Owing to the fact that but few studies have been made on the fleas of the genus *Rhopalopsyllus* that infest the wild animals of Panama and other Central and South American countries they should be considered as potential transmitters of plague among rodents and other small animals.

The black rats, *M. rattus,* which are usually semi-domesticated in their habits have also become naturalized in the open country in Panama to a considerable extent and are therefore not only present in the houses in proximity to man, but are also found at the outskirts of towns and cities as well as in the jungle at considerable distances from habitations, where they have practically acquired the habits of a wild species. The brown rat, *M. norvegicus,* has also taken to the bush to some extent, being found along the banks of the Canal at a distance from houses and evidently receiving plenty of food from refuse thrown overboard from ships while passing through the Canal, but apparently has not adapted itself to the open country in the same manner as the black rat. These species of semi-domesticated rats that are known to be susceptible to plague are therefore not only living in close proximity to man but are also in contact with the bush species of rats in the jungle, such as the cotton rats, *S. h. chiriquensis,* and the spiny rats, *P. s. panamensis,* both of which have been found acting as hosts for fleas of the genus *Rhopalopsyllus,* and many other varieties of rats that are likely to be infested with fleas of various species. It is therefore evident that if plague were introduced in Panama and became at all prevalent among the rats in the cities and towns it would be very apt to spread outside and become endemic among the rats in the jungle. This would create a situation not only infinitely more difficult to control than that existing in California where plague persists endemically among the ground squirrels, *Citellus beecheyi,* but one that would be extremely dangerous to the population of Panama.

RESÚMEN

La importancia de las pulgas depende del papel importante que estos insectos desempeñan en la transmisión de las enfermedades.

Xenopsylla cheopis Rothschild

Pulga de las ratas de India. Constituye el 97.9 por ciento de las que se encuentran en las ratas de Panamá. Durante varios años se han venido introduciendo medidas preventivas en contra de las ratas tales como la protección o construcción especial de viviendas viejas y nuevas y el uso de hormigón para muelles y astilleros.

Este artículo dá estadísticas extensas sobre la predominancia de las pulgas de ratas y ratones en 1909 y 1910 y también de 10 años más tarde.

Dermatophilus penetrans Linnaeus

Prevalece en muchas de las aldeas nativas. El cerdo es su huésped favorito aunque también ataca a otros animales incluyendo al hombre. La hembra preñada perfora y se introduce dentro de la piel del huésped en donde permanece hasta que desarrolla y deposita los huevos. Es la causa de mucho dolor e incomodidad particularmente de los pies a los que con más frucuencia ataca.

Pulex irritans Linnaeus

La pulga humana. Muy freceunte en Panamá. El hombre es el huésped favorito.

Echidnophaga gallinaceous Westwood

Pulga de las gallinas. Presente en varias localidades de Panamá. El huésped favorito es la gallina pero se encuentra a veces en otras aves y en perros, gatos y ratas. Tambien horada la piel y es probable que así deposite los huevos.

Ctenocephalus felis Bouche

La pulga de los gatos. Muy prevalente en Panamá.

Ctenocephalus canis Curtis

Encontrada una sola vez en un perro de Panamá.

Rhopalopsyllus dunni Jordan and Rothschild

Encontrada en las ratas algodoneras de Panamá.

Rhopalopsyllus klagesi Rothschild

Encontrada en la rata espinosa de Panamá.

Rhopalopsyllus cacicus Jordan and Rothschild

Se encuentra en el oso hormiguero.

El género *Rhopalopsyllus* debe considerarse como el transmisor potencial de la plaga (peste) entre los rodentes y otros pequeños animales. En Panamá ambos *M. pattus* y *M. norvegicus* han abandonado hasta cierto punto la morada del hombre y se han habituado a la vida al campo libre. De esta manera mantienen contracto entre el hombre y su domicilio y las especies de ratas de matorral (algodonera, espeinosa, etc.). Por lo tanto, si la peste se introdujera en Panamá y prevaleciera entre las ratas de la ciudad es probable que la enfermedad se hiciera endémica entre las ratas de las malezas. Esto crearía una situación de mucho más dificil control que la de California, por ejemplo, en donde la plaga es endémica entre las ardillas del pasto.

REFERENCE

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THE TROPICAL BEDBUG IN RELATION TO THE TRANSMISSION OF RELAPSING FEVER IN PANAMA

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In 1921, the writer, while Entomologist at the Board of Health Laboratory, Ancon, Canal Zone, began a series of experiments to determine the relation of the tropical bedbug, *Cimex rotundatus* Signoret, to the transmission of relapsing fever in Panama. However, owing to his departure from the Canal Zone soon after beginning this work only a few of these experiments were completed. These consisted of biting experiments, inoculations with suspensions of macerated bedbugs and examinations of smears made from fluids and dissected parts of bugs, a report of which is given herewith.

BITING EXPERIMENTS

Biting experiment 1: Bugs fed on human case of relapsing fever and later fed on white rat

March 26, 1921. Fifty adult bedbugs were placed on H. C., a relapsing fever patient in Ancon Hospital, whose blood contained spirochetes in scant numbers. These bugs had not been fed for about fifteen days previously and 49 of them readily engorged when given this opportunity. From March 28 to April 4, inclusive, these 50 bugs were daily placed on a shaven area on white rat 1 and the number that fed each day averaged 88 per cent, making a total of 704 bites that the rat received from this lot of bugs. Blood films from this rat were examined (600 fields each day from March 29 to April 15 but no spirochetes were found. The rat was killed and autopsied April 16 but nothing was found to indicate that it might have had relapsing fever. No dissections were made of this lot of bugs.

Biting experiment 2: Bugs fed on rat infected with relapsing fever and later fed on monkey

April 26, 1921. One hundred bugs were fed on infected white rat 360 whose blood stream contained numerous spirochetes, there being from 1 to 3 present in each field when examined microscopically with a Bausch and Lomb $\frac{1}{12}$ oil immersion objective and 5× eyepiece. These bugs were all adults and as none of them had fed within eighteen days prior to this feeding they all engorged very rapidly. Adult bugs were used for this experiment as it was considered that they would take blood more regularly if given the opportunity for feeding daily than the immature stages would, as the latter usually do not feed for one or two days before each molt. It was also believed that there would be more excreta from the adults, especially the males, than from the immature stages, owing to the development of the latter, and it was desired that there be as good an opportunity as possible for observations on the excreta as a factor in the transmission of the fever.

April 27, 1921. Sixty of these bugs were placed on a practically bare area at the inguinal region of monkey 67, a *Macaccus rhesus* from the Old World. Fifty-six of the bugs readily engorged. One of the four that did not feed was dissected and several spirochetes found in the gut contents and salivary glands.

April 28, 1921. This lot of bugs was again placed on the monkey and 50 of them fed, making 106 bites that monkey 67 received from the bugs that had fed on the infected rat.

The temperature of this monkey was taken and its blood examined (800 fields) daily from April 27 to May 20, but there was no appreciable increase of temperature and no spirochetes were found.

Biting experiment 3: Bugs that had fed on rat infected with relapsing fever and with two subsequent feeds of uninfected blood, fed on monkey thirty-five days later

May 31, 1921. Eight bugs of the lot that had fed on infected rat 360 on April 26 and on uninfected monkey 67 on April 27 and 28 (biting experiment 2) were placed on monkey 68. It was thirty-five days since these bugs had fed on infected blood and thirty-three days that they had been without any feeding and all of them engorged very rapidly when placed on this monkey. Four of these bugs were dead the following day but the remaining 4 were placed on this monkey each day to feed during the next nine days.

The feeding record of this experiment is as follows:

May 31, 1921.	Eight fed	June 1, 1921.	Four fed
June 2, 1921.	Four fed	June 3, 1921.	Four fed
June 4, 1921.	Three fed	June 5, 1921.	Four fed
June 6, 1921.	Three fed	June 7, 1921.	Two fed
June 8, 1921.	Four fed	June 9, 1921.	Four fed

The above makes a total of 40 bites that this monkey received.

Daily temperatures and examinations of blood films from this monkey from May 31 to June 20 failed to show any evidence of relapsing fever.

During these biting experiments it was observed that there was practically no excreta deposited on the skin by the bugs. They usually returned to the wall of the tube in which they were confined while feeding before excreting.

INOCULATION EXPERIMENTS; SUSPENSIONS OF MACERATED BUGS INJECTED INTO ANIMALS

Contents experiment 1

May 11, 1921. Twenty-five bugs, 12 males and 13 females, that had fed on infected rat 360 on April 26 and on uninfected monkey 67 on April 27 and 28, this giving two meals of uninfected blood subsequent to feeding on infected blood, were macerated in 1 cc. of normal saline solution and injected into white mouse 335. Part of this suspension was injected intraperitoneally and part subcutaneously.

Contents experiment 2

May 28, 1921. Fourteen bugs, 5 males and 9 females, that had fed on infected rat 360 on April 26 and also on uninfected monkey 67 on April 27 and 28, were macerated in 1 cc. of normal saline solution and injected intraperitoneally and subcutaneously into white mouse 344. As these bugs had been last fed on April 28 they had been thirty days without feeding and thirty-two days since their last meal of infected blood. Spirochetes were found in the blood of this mouse forty hours after the bugs were injected.

TABLE 1

Examination of blood films from white mouse 335

DATE	RESULT	NUMBER OF FIELDS EXAMINED
1921		
May 12	Negative for spirochetes	600
May 13	Negative for spirochetes	600
May 14	*Positive for spirochetes*	Average 1 in 19 fields
May 15	*Positive for spirochetes*	Average 1 per field
May 16	Negative for spirochetes	600
May 17	Negative for spirochetes	600

Contents experiment 3

June 10, 1921. Four bugs that had fed on infected rat 360 on April 26 and on uninfected monkey 67 on April 27 and 28 and then remained unfed until May 31, when they fed daily from that date until June 9 on monkey 68, were macerated in 1 cc. of normal saline solution and injected intraperitoneally and subcutaneously into white mouse 382. These bugs were the ones used in biting experiment 3. Examinations of blood films from this mouse were made daily from June 11 to June 26 but no spirochetes were found.

The two monkeys (nos. 67 and 68) used in the foregoing experiments were both infected with the same strain of relapsing fever at a later date, one being infected through bites of the human tick, *Ornithodoros talaje,* and the other by an injection of infected blood. This was done in order to test the susceptibility of these particular monkeys to relapsing fever.

EXAMINATIONS OF SMEARS MADE FROM DISSECTED BEDBUGS

The bugs used in these dissections had fed on infected rat 360 on April 26 and on uninfected monkey 67 on April 27 and 28. The coelomic fluid was obtained for examination by pulling legs from the bugs and making smears of the fluid which exuded.

Dissections made two days after feeding on infected blood

April 28, 1921. Three bugs were dissected about six hours after feeding on uninfected blood and forty-eight hours after a meal of infected blood. Numerous spirochetes were found in the smears made from the gut contents and salivary glands from 2 of the bugs, while the third showed but one and that in the smear from the gut contents.

TABLE 2

Examination of blood films from white mouse 344

DATE	RESULT	NUMBER OF FIELDS EXAMINED
1921		
May 29	Negative for spirochetes	600
May 30	*Positive for spirochetes*	Average 1 in 167 fields
May 31	*Positive for spirochetes*	Average 1 in 235 fields
June 1	*Positive for spirochetes*	Average 1 in 11 fields
June 2	*Positive for spirochetes*	Average 1 in 316 fields
June 3	Negative for spirochetes	600

Dissections made eight days after feeding on infected blood

May 4, 1921. Five bugs were dissected and many spirochetes were found in smears of the coelomic fluid from 3 of them, none being found in the other 2. No spirochetes were found in the gut contents or salivary glands of any of these bugs.

Dissections made fifteen days after feeding on infected blood

May 11, 1921. Nine bugs were examined and 3 found positive, spirochetes being present in the coelomic fluid in scant numbers. None were found in the gut contents or salivary glands.

Dissections of bugs made at later dates failed to show any spirochetes.

It is to be hoped that further experiments may be carried out in connection with this work in order to more definitely determine the possibility of bedbugs transmitting relapsing fever in Panama by means of their bites.

SUMMARY

Bedbugs that had fed on man and on rat infected with relapsing fever did not transmit it to uninfected animals by feeding on them at subsequent intervals.

Bedbugs caused relapsing fever when macerated and inoculated into a white mouse as late as thirty-two days after they had fed on an infected animal.

Spirochetes were found in the coelomic fluid of bedbugs fifteen days after feeding on infected blood. This would indicate that the spirochetes survive and apparently multiply in the bugs for a while at least.

SUMMARIO

Cuando la chinche se pone en contacto con el cuerpo de hombres y ratas infectadas con fiebre recurrente, de manera que los insectos obtengan alimentación por chupadura directa, no se consigue transmitir la enfermedad al hacer que las chinches así alimentadas piquen subsiguientemente a otros animales sanos.

Las chinches causaron la enfermedad después de haber sido maceradas e inoculadas a un ratón blanco habiendo transcurrido un intervalo de 32 días desde el contacto de aquellas con el animal infectado.

Encontramos espiroquetes en el fluído celómico de las chinches a los quince días después de la alimentación de aquellas con sangre infectada. Esto parece indicar que los espiroquetes sobreviven y aparentemente se multiplican por algún tiempo en las chinches.

A NEW SPECIES OF ANOPHELES IN LOUISIANA[1]

ANOPHELIS ATROPOS D. & K.

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The continuation of the mosquito and malarial survey and control work, begun in September of last year, 1921, was again taken up this June. As the coast section of the State had never been systematically exploited in this connection, it was originally the intention to investigate the mosquito and malarial conditions of Terrebonne, St. Mary, Vermillion and Cameron Parishes. For certain reasons which will become evident later on, the work had to be confined to St. Mary and Terrebonne.

It is, of course, needless to repeat here that the prevention of disease dissemination by insects depends upon the eradication of the disseminators. This, in turn, implies that the latter should be known specifically as well as for the individual habits of each one.

While primarily engaged in conservation work during the early spring of 1901, the writer incidentally found a species of Anopheles on Terrebonne Bay which appeared unusual to him on account of its having unspotted wings. Search for a description proving fruitless, the mosquito was reported to the Bureau of Entomology in Washington as an undescribed species. Subsequently it was discovered that the species had been found by Gibson in Ottawa, Ontario, just a few months previously (August 23, 1900). The writer's specimens, sent on to Washington, were dated March 29, 1901. The species was described by Theobald under the name of *Anopheles Walkeri*.

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[1] No. III of Mosquito and Malaria Survey of Louisiana State Board of Health. Illustrated by XV plates and 27 figures, only two of which are here reproduced.

Knowledge on the transmission of diseases by mosquitoes was, at that time, still in infancy, and it was in connection with his pioneer work along these lines that the writer had paid any attention at all to this new species of Anopheles. Eventually, correspondence with Washington was discontinued because no further data on the subject had been forthcoming.

Some time during the spring of this year a large number of mosquitoes were sent to this office for identification from Gulfport, Mississippi. Two of these mosquitoes were badly mutilated females of *Anopheles Walkeri.* A request for more material and data was issued immediately but up to date without result.

In the meantime, a new angle presented itself in so far, that in the course of twenty-one years no further reports had been received on the species, at least, not from the south, but that its discovery so far east from its originally reported locality seemed to indicate an enlarging breeding territory. Furthermore, the mosquito being an Anopheles, rendered it likely to be a malarial vector as well. Its life cycle, its larvae and pupae and even the males, being unknown, its capacity of malarial vestorship equally as obscure, it became, at once, a salient question in connection with malarial control.

For this reason it was deemed advisable to revisit the original locality for the purpose of determining its prevalence, extent of distribution, and, above all, to become acquainted with its life cycle and the rôle it might play in the transmission of malaria.

The boat Opelousas of the Louisiana State Conservation Commission having been kindly tendered to the writer by the Commissioner, Mr. M. L. Alexander, the start was made from Morgan City for Terrebonne Bay on June 5, arriving the same evening at the mouth of Terrebonne Bayou.

The route traversed from the mouth of the Atchafalaya to Terrebonne was through a now almost uninhabited region of larger and smaller islands, lifeless, treeless and desolate in the extreme. Even the fisher people, who formerly populated this region, have deserted it and have moved their families from thirty to forty miles inland and out of the reach of ever-threatening storms and tidal-waves. Extremely fertile and productive, well settled and cultivated sections were rendered barren in a single night, and have remained so since the last storm which struck that region in September, 1909.

A straight line drawn from Buras on the Mississippi due west to the mouth of the Atchafalaya, a distance of about 85 miles, would leave that section of the State south of that line practically without any permanent habitation or settlement and, therefore, also non-malarial. North of this line the first settlements begin to appear, Cocodrie on little Caillou, Boudreaux, Chauvin and Montegut. North of these, however, there is another straight belt which is practically uninhabited and comprises about fifteen parish sections. From there on northward the population and permanent communities rapidly increase, with Houma, the southernmost town in the State.

South of that point control work is, naturally, out of question. The only permanent residents of all that marshy region south of the line mentioned, are with the exception of a few species of birds, mosquitoes, among which, however, race-suicide appears to have no standing.

From information gathered from several people it appears that before the catastrophe of 1909, some of the settlements on the banks of the bayous and lakes used to suffer quite regularly from malarial infections.

At the present time it is rather difficult to obtain any information. Most of the class of people in that section are, naturally, very illiterate, and, for that reason extremely suspicious of the motives which a stranger might have for asking questions.

On the morning after tying up to the shore and even before daybreak, the cabin of the boat was swarming with mosquitoes, only three species, however, could be identified: *Anopheles Walkeri*, *Anopheles crucians* and *Aedes sollicitans*. Of these the first one was, by far, the most numerous and observations on its habits and other characteristics were begun at once.

ANOPHELES ATROPOS D. & K.

In the course of the very close study and analysis of over one hundred perfect specimens, it soon became evident that the mosquito under consideration and referred to in the preceding pages under the name of *A. Walkeri*, differed from the descriptions of that species by Theobald and Howard, Dyar and Knab to such an extent that the conviction of its not being the same species became fully confirmed. Furthermore, these studies clearly showed it to conform in most respects with *A. atropos*, described by Dyar and Knab in the "Proc. Biol. Soc. Wash., xix, 160, 1906."

The specimens secured in Terrebonne Parish under date of March 29, 1901, and sent to the Bureau of Entomology, were, at that time, identified and recorded under the name of *A. Walkeri*, Theobald. This mistaken identification was, no doubt, due solely to lack of sufficient and perfect material.

In the meantime, all subsequent descriptions and discussions of this mosquito in this paper will be provisionally under the name of *Anopheles atropos* D. & K.

General appearance and habits

In its general appearance *Anopheles atropos* is a very dark, almost black insect and it is only when viewed closely and in strong light that its coloration becomes distinct. Its normal resting position is distinctly different from that of *A. crucians* and *A. quadrimaculatus* as well as from that of Culex, Aedes etc. When resting on a vertical surface the body of Culex is parallel to it, in *A. crucians*, etc., it is at nearly a right angle. *A. atropos* holds its body at an angle of forty-five degrees and, usually rests on five legs instead of four.

In its attacks upon man it is direct and vicious, and almost as pertinacious as *Aedes sollicitans*, preferring to be killed with a full stomach, rather than to fly to safety with an empty one.

After catching and killing practically all of the mosquitoes on the boat, a quest for the breeding places was made on shore, which, according to the uniform topographic features for miles and miles around, had been expected to be plentiful. Large and small puddles of water were carefully searched in all directions without avail. Not a single larva or pupa could be found, no matter how much every detail was scrutinized. Then it

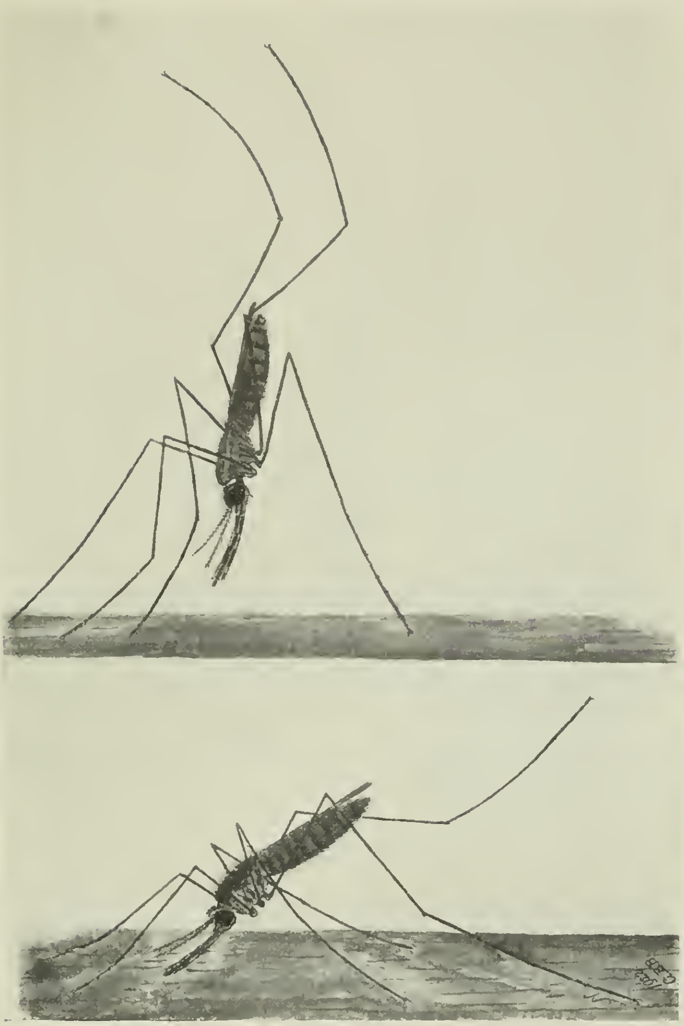

FIG. 1

became evident that there were no algae of any kind in the places which had been examined, and the search was made for these plants. None, however, could be found, but their absence was accounted for by the high water which had inundated that section.

While searching for the larvae in the water and among the grasses and other water plants, no mosquitoes except *Aedes sollicitans*, could be noticed. Sitting among the tall grasses drew no Anopheles. But no sooner had the boat been boarded again when hundreds of the Anopheles were encountered in the cabin and pilot house. Evidently they had invaded the boat while the quest for them was being made on land. Where had they come from, especially at that time of the day? The same puzzling experience was encountered afterwards wherever a stop was made. No *Anopheles atropos* were captured on the entire trip anywhere but on the boat. Furthermore, among the hundreds killed and captured in day time, there was but one male which, unfortunately, escaped capture. This fact clearly indicated that the breeding places were not nearby where they had been looked for. Later on, while visiting one of the houses in Cocodrie after dark several atropos females were seen inside.

Soon after dark it was impossible to stay anywhere except under the mosquito net, and the nights were simply hideous. Even a heavy squall one night failed to make any impression upon the countless thousands buzzing outside the netting. In the entire region visited on this trip, only three species of mosquitoes were noticed, and the ratio between them appeared to be: that for every hundred of *Anopheles atropos*, there were seventy-five *Aedes sollicitans* and twenty-five *Anopheles crucians*.

Description

Anopheles atropos is somewhat smaller than either *A. quadrimaculatus* or *A. crucians*, measuring about 8 mm. from the tip of the proboscis to the end of the abdomen. In color the sexes are alike. The head is grayish-brown, the eyes are black, broadly bordered with whitish scales. The proboscis is dark brown. The palpi are dark brown with a pale, indistinct ring encircling the articulation between the second and third joints in the female only. There is no ring on the palpi of the male. The color of the thorax is somewhat variable in both sexes, sometimes chestnut brown but more usually dark brown, covered with long dark brown hairs which in strong light reflect almost golden.

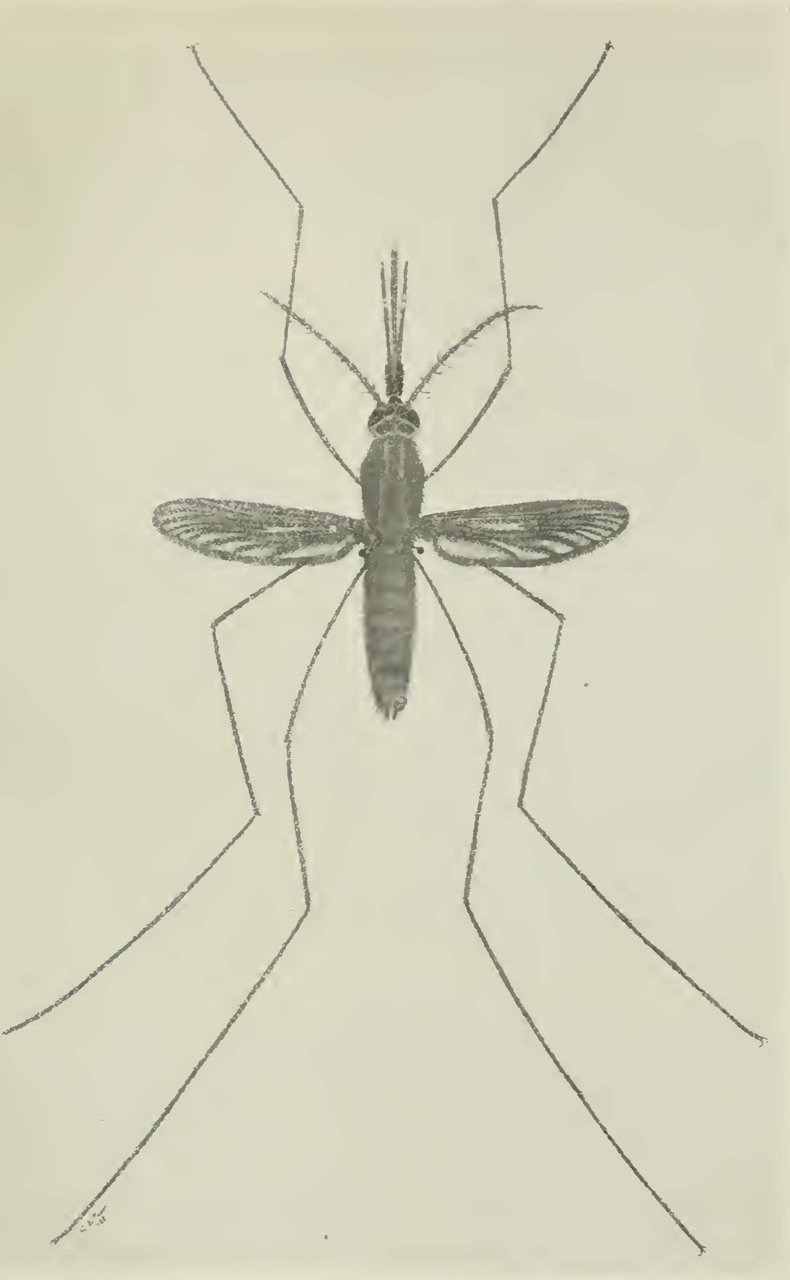

FIG. 2

The abdomen is dark brown, irregularly mottled with black, some few specimens show faint traces of pale segmental bands. The vestiture consists of long dark brown hairs. The wings are moderately long but broader than in *A. quadrimaculatus* or *A. crucians*, they are unspotted though a few individuals (females only) show a slight massing of scales on the forks of the veins. The legs are long and slender, clothed with dark brown scales and hairs. The description in detail of both sexes as well as that of the larvae at different stages and the illustrations (14 plates, 26 figures) are contained in the original report but have been omitted in this article. They may be examined upon request in the Board's office.

Having now described the adult insects it may not be out of place to state briefly wherein *A. atropos* differs from *A. Walkeri* with which it was confounded. At the time, when Theobald described the specimens from Ottawa, Ontario and Lake Simcoe as a new species under the name of *A. Walkeri*, atropos was not as yet known, and, when then the first specimen from Terrebonne Bay was sent in, it was, on account of its great superficial resemblance supposed to be *A. Walkeri*. Again, when Dr. Hiram Byrd of Jacksonville, Florida, sent his specimens from the Florida Keys to Washington, Dyar and Knab promptly recognized the specific differences between the two mosquitoes and called the Florida form *A. atropos*. But the real and first discovered *A. atropos* of March 29, 1901, from Terrebonne Bay was, in the meantime, put away under the wrong name of *A. Walkeri*.

There are, at the present writing, two descriptions of *A. Walkeri*, and two of *A. atropos*, available. For the former the original description of Theobald (monograph of the Culicidae) and that of Howard, Dyar and Knab ("Mosquitoes of North America"), for the latter, the original description of Dyar and Knab 1906 which, however, is entirely unsatisfactory, and the better and far more correct one, also contained in the "Mosquitoes of North America." The comparisons are based entirely upon the last named description.

The differences between the females of the two species in tabulated form are as follows:

Anopheles Walkeri, Theob.	*Anopheles atropos.* D. & K.
Palpi with dull, silvery-white rings at at the middle of long joint, at the articulations of the last two joints and the tip of the last one.	Palpi with pale, silvery scaled ring around articulation between second and third joint but none anywhere else. Tip of last joint dark. In many specimens even the ring is obsolete.
Vestiture of occiput of erect pale scales, their stems dark, some slender recumbent whitish scales anteriorly, a tuft of long, white setae projecting forward between the eyes.	Vestiture of occiput and vertex of erect, triangular black scales, no slender recumbent white scales anteriorly. Tuft of setae between the eyes black.
Prothoracic lobes with brown bristles. Vestiture of Mesonotum of short, sparse, golden-brown hair-like scales, bristles at root of wings coarse, and brown. Postnotum pale, brownish-grey.	Prothoracic lobes with black bristles. Vestiture of Mesonotum of dark brown, rather long, hair-like scales with yellowish reflections. Bristles at root of wings coarse and black. Postnotum brown with whitish pruinose centre.
Abdomen brownish-grey, slightly rugose with numerous, short brown bristles with golden luster.	Abdomen brown with black, irregular blotches. Vestiture of rather long, dark brown nearly black hairs with golden-yellow reflections.
Wings hyaline, petiole of second posterior cell about as long as its cell.	Wings slightly smoky, petiole of second posterior cell longer than its cell.
Halterers brownish. Legs long and slender, vestiture blackish-brown.	Halterers pale, knob black. Legs with black scales and deep blue reflections.
Male not known, or, at least, description not published.	Male as described in preceeding pages.

Its reproduction

The first females were caught early in the morning of June 5, and some were added every day until the morning of the 8th. The majority of those which were intended for breeding, were allowed to fill themselves with blood before they were turned loose in a large wire cage. Most of those which had not been fed, died off after twenty-four hours, some lived a day longer, but, by the time the laboratory was reached only the blood-fed ones seemed to have survived the journey. A lump of moistened sugar suspended in the cage, had however, been maintained from the beginning. The first eggs were deposited on June 10, presumably by the females fed on the 5th. There was a delay in oviposition which should have occurred within, at least, seventy-two hours after the meal of blood.

The egg

The egg resembles that of *A. quadrimaculatus* in shape as well as in the distribution of the floating membrane. The time for hatching, however, differs very materially from that required by either *A. crucians* or *quadrimaculatus* where the average time is about seventy-two hours. The earliest larvae of *A. atropos* issued within forty and the latest after not more than forty-eight hours.

There is every indication that the eggs are capable of withstanding dessication, at least, for some time, for if any of those which adhered to the sides of the jar were left above the water level by evaporation, some, if not all of them, hatched the moment they came again in contact with the water. Moreover, recent laboratory experiences indicate that in freedom they are deposited on the wet mud and not in the water as it is the case with *Anopheles crucians* and *quadrimaculatus*. Recently a number of females were placed into a large salt mouth jar, the bottom of which had been covered with a moistened cloth. A few hours later several hundred eggs had been laid on the cloth. A like number of females had been put into a jar containing about 3 inches of sea water, but they did not lay until four days later. All females concerned in these two observations had been blood-fed within a period of not more than an hour. The inference here is permissible that the first set found the conditions for oviposition more suitable than the second set which delayed it because they were not.

In the beginning several obstacles were encountered in raising the larvae. The causes for the failure were due, partly, to the want of sea water, partly to lack of proper food and, partly to the presence of voracious larvae of other insects. Even after the latter had been eliminated by boiling the algae and other bits of vegetable tissue, hardly any growth took place.

In the meantime the observation in regard to the unexpected oviposition on wet cloth had been made and the surmised mud-breeding proclivity was given the test. In order to provide more food than that which had been brought with the mud from the marshes, a quantity of *Potamogeton pusillus* and *Cladophora fracta* was boiled for about twenty minutes. When this had cooled, it became covered with a slimy scum and simply smelt to the heavens. On microscopic examination the larger proportion of the scum was found to consist of a viscid slime and hundreds of Vorticella. Some of the marsh mud was now spread on the bottom of a shallow glass dish and small quantities of the boiled food were scattered about. Only enough sea water was used to barely cover the mud. The larvae which were then about forty-eight hours old and did not measure 1 mm., were put into the dish. From that moment on they fairly revelled in the mud, tearing away at the slimy scum, shaking off the surplus and swallowing quantities apparently all out of proportion to their size. Their growth, consequently, was fairly proportionate to their voraciousness. The sum of what has been or will be mentioned in detail in connection with the development and habits of the larvae, may as well be stated now. From eggs which had hatched during the night of September 9 and 10, the first adults emerged on the morning of September 24. In other words there was a difference of twenty-two days between this period and the first attempt. It may be assumed that this period of development corresponds very likely to what normally occurs in freedom. Unlike *Anopheles crucians* or *quadrimaculatus, A. atropos* requires, at least, one week less time for its reproduction than either of those two sepcies.

The larvae are almost exclusively mud denizens and feeders, in order to thrive the volume of water must be reduced to a minimum, and only when they are replete with food will they assume what is usually believed to be the characteristic position of all Anopheline larvae. Furthermore they seem to be gregarious and prefer to congregate in groups, for on being separated by stirring up the mud, they will be found again collected into groups of eight or ten individuals in an hour's time or less; this will be repeated as often as they are separated.

When first hatched the larva measures less than 1 mm., it is quite colorless and transparent with the exception of its large head which is dark. Gradually, the color deepens and at the end of the third day it sheds its first skin and becomes dark brown with white, transverse bands. These cross bands consist really of large white spots, usually two on each segment. The broad band on the anterior part of the thorax is made up of four spots. In this conspicuous coloration the larva remained until after the second moult which occurred approximately three days after the first. The color now changed to dark gray, the large white spots were no longer as conspicuous and the majority of them seem to have dissolved into minute white dots, dispersed over the entire dark median area of each segment. After the third moult the large spots disappear completely and only the minute ones remain, making the larva almost mud colored and rather hard to distinguish from its surroundings. After the fourth ecdysis the larva is ready for the last pre-adult stage.

When full-grown the larva measures from 8 to 8.5 mm. Its color is then gray or yellowish gray with a dark median stripe.

The pupa

Cephalothorax subpyriform, almost nude. Siphons long, funnel-shaped, deeply cleft on inner side and widely flaring. Abdomen stout, the last three segments depressed; hairs very sparse, short, bifid and branched on dorsal side of segments; on posterior margin hairs long and branched; the apical angles with a short, stout, pointed spine. The first abdominal segment bears a pair of highly developed, palmately-cleft hairs resembling the fan-shaped tufts of the larva. Anal paddles longer than last abdominal segment, about four-fifths as broad as they are long and terminated by a short, branched seta.

The distribution of this species of Anopheles seems to be entirely littoral and restricted to a very narrow belt along the gulf coast. It was reported from the Florida Keys by Dr. Hiram Byrd, from Buras, La., by Dr. M. J. White, and from Gulfport, Miss., by the writer. Quite recently it was found as far west as Fresh-water Bayou, Vermillion Parish, but was not noticed on the shores of White Lake, five miles north of the mouth of the bayou. There are apparently no records of its occurrence along the North Atlantic coast.

In connection with the life of the adult insects there are several phases which remain unexplained so far. In the first place, where do they actually deposit their eggs? As already related, laboratory breeding experiments showed them to be mud feeders in their larval stage. On both excursions into their breeding grounds, almost every foot of land was under water to the depth of 6 inches and more, and yet no larvae could be found anywhere, but the adults swarmed in countless numbers.

Second, where do the adults keep themselves during the day time, why could none be seen 6 feet away from the boat while at the same time they were gathering on board in broad sunlight?

Ascending Bayou Terrebonne, the adults (females only) came aboard the boat as far as ten miles inland, when they were suddenly replaced by *Anopheles crucians* and *quadrimaculatus*. What is the reason for this sharply drawn limit? High tide or pure salt water reaches considerably beyond this ten mile limit at times.

Compared with other Anopheles, *atropos* seems to have little or no power of endurance outside the limits of its habitat. Unlike most species of Anopheles, it can apparently not endure confinement. On this account all efforts of determining its vectorship of malaria were rendered futile because no females could be kept alive long enough to be fed on malarial patients. Only those which had been blood-fed at capture survived and reached oviposition.

Indirect, and, therefore, questionably reliable evidence, however, indicates that this species is capable of carrying malarial infections, and, of course, efforts to establish a positive knowledge on this important point are still being made.

BOOK REVIEW

Alphonse Laveran, sa Vie, son Oeuvre. By MARIE PHISALIX, Agrégée de L'Enseignement Secondaire des Jeunes Filles; Docteur en Médecine de la Faculté de Paris. Paris: Masson et Cie., Éditeurs. Librairies de L'Académie de Médecine, 120 Boulevard Saint-Germain (6e), 1923.

The paper bound volume, Alphonse Laveran, sa vie, son oeuvre, is an interesting and instructive résumé of the life and work of the discoverer of the malarial parasite. It should never be forgotten that this organism was found by Laveran, who was at that time a surgeon in the French Colonial Army, stationed in Algeria, using a microscope of which the highest power was given by a $\frac{1}{6}$ inch objective. In the second edition of his *Traité du Paludisme,* Laveran thus describes the manner in which the organism was found. "My first studies go back to 1878. I was at that time in charge of a service in the hospital at Bône (Algeria), and a large number of my patients were suffering from malarial fever. I had the opportunity of making autopsies on several subjects dead of pernicious malaria and of studying the melanemia, which had already been observed, but which was not considered as a constant change in malaria, nor as a special characteristic of that disease. I was struck by the singular characters of the black pigment granulations, particularly in the liver and in the cerebral vessels, and I endeavored to pursue the study of the formation of the pigment in the blood of patients suffering from malarial fever. In the blood I found leukocytes containing pigment, already seen by other observers, but, in addition to the pigmented leukocytes, my attention was drawn to spherical pigmented bodies of variable volume, endowed with ameboid movements, and pigmented crescent-shaped bodies. I supposed at that time that they were parasites. In 1880, at the military hospital of Constantine, I discovered on the edges of these spherical pigmented bodies, in the blood of a patient suffering from malaria, filiform elements resembling flagella, which moved with great quickness, displacing the neighboring red corpuscles; from that time I had no doubt of the parasitic character of the elements which I had found in malarial blood. Further studies showed me that when studied under favorable circumstances (during the paroxysm and before the use of quinine) these parasitic bodies were always found in the blood of patients with malaria, and that they were never found in other diseases. I arrived, thus, at the conviction that they were indeed the parasites of malaria."

But Laveran was a worker in other fields than that of malaria. He worked on the trypanosomes, gregarines, sporozoites, leishmania, and spirillae. One of his chief accomplishments was the establishment of the Laboratory of Tropical Diseases in the Pasteur Institute at Paris (1906–1907), where he worked until his death in 1922.

The first chapter of the book under review is devoted to a biographical sketch of Professor Laveran and contains a reproduction of the inscriptions on the commemorative tablets placed in the room in the military hospital of Constantine, which he used as a laboratory. These tablets were placed in 1908: One, erected by the civil physicians of Algiers, has the following inscription:

M. LAVERAN
MÉDECIN—MAJOR DE 1ER. CLASSE
A DÉCOUVERT L'HÉMATOZOAIRE DU PALUDISME
EN 1880

The other, erected by the military physicians of the Constantine Hospital, reads as follows:

A

ALPHONSE LAVERAN
MEMBRE DE L'ACADÉMIE DE MÉDECINE, 1893
MEMBRE DE L'INSTITUT, 1901
PRIX NOBEL DE MÉDECINE, 1907
QUI, DANS CET HOPITAL
A DÉCOUVERT L'HÉMATOZOAIRE DU PALUDISME
EN 1880

Hommage respectueux des Médecines Militaire de Constantine,
20 Aôut, 1908.

Chapter II deals with his work on malaria; chapter III with the works on intracellular hematozoa other than the malarial parasites; hemamebae, piroplasmata, hemogregarines and toxoplasmata. Chapter IV deals with work on the sporozoa; chapter V with Leishmania and Leishmaniasis; chapter VI with studies on herpetomonas and crithidia; chapter VII with trypanosomes and trypanosomiasis; Chapter VIII with works on the bacteria, spirilli and fungi; chapter IX with studies concerning pathology and pathologic anatomy; chapter X with physiology and experimental pathology; and chapter XI with works on hygiene. This chapter is followed by a list of the publications of M. Laveran, containing nearly 600 titles. The book contains many full quotations from Laveran's more important papers; and two excellent pictures. One of these is by E. Boiloin, etched in 1894; the other is a photograph made by Pirou in 1901, with a reproduction of his signature.

M. Laveran died in 1922, at the age of 77 years. The volume is a fitting tribute to the life and accomplishments of one of the great men of medicine.

John M. Swan.

457 Park Ave., Rochester, N. Y.

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TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE, NINETEENTH ANNUAL MEETING, HELD AT SAN FRANCISCO, CALIFORNIA, JUNE 25 AND 26, 1923

THE AMERICAN SOCIETY OF TROPICAL MEDICINE

The American Society of Tropical Medicine was established March 9, 1903. It has been chartered as a perpetual corporation by the State of Pennsylvania. It is one of the component organizations of the Congress of American Physicians and Surgeons. Its purpose is to advance the knowledge of tropical diseases by encouraging original research by its members and others; collecting and recording facts ascertained by such researches, and disseminating information thereof by discussion among its members and by the publications of papers read before the Society. The affairs of the Society are administered by a Council of five, a President and a Secretary, elected by the Society. Any regular American physician or scientist interested in the study of tropical diseases is eligible for Active Membership. Any regular physician or scientist not a resident of the United States, who has contributed to the knowledge of tropical diseases is eligible for Corresponding Membership. The number of active members is limited to 300. Candidates for membership are elected by ballot at the annual meeting upon recommendation by the Council. The dues are fixed each year by the Council but can not be increased beyond $5.00 without approval by the Society at its annual meeting. The official journal of the Society is the AMERICAN JOURNAL OF TROPICAL MEDICINE (bi-monthly) which goes without additional cost to all active and corresponding members paying annual dues.

ARTICLES OF ASSOCIATION

CHARTER

Be it known that the subscribers, having associated themselves together for the purpose hereinafter mentioned, and being desirous of becoming incorporated agreeably to existing laws of the Commonwealth of Pennsylvania providing for the incorporation and regulation of certain corporations, approved April 29, 1874, and its supplements, do hereby declare and certify that the following are the purposes, objects, articles, and conditions of their said association, for and on which they desire to be incorporated:

I. The name of the corporation shall be: American Society of Tropical Medicine.

II. The purpose for which the corporation is formed is to advance the knowledge of tropical diseases by encouraging original research by its members and others; collecting and recording facts ascertained by such researches, and disseminating information thereof by discussion among its members and by the publication of papers read before the society.

III. The corporation is to exist perpetually.

IV. The location of the Secretary's office shall be in the city of Philadelphia.

V. The names and residences of the subscribers hereto are as follows: Thomas H. Fenton, M.D., 1319 Spruce St., Philadelphia; Judson Daland, M.D., 317 South 18th St., Philadelphia; Joseph McFarland, M.D., Medico-Chirurgical College, Philadelphia.

VI. The corporation is to be managed by a Council of five members, and a President and Secretary, who shall be ex-officio members of the Council. In addition thereto there shall be annually chosen a Treasurer, two Vice-Presidents, and an Assistant Secretary. The names and residences of those chosen for the first year are: Thomas H. Fenton, M.D., Philadelphia, President; James C. Wilson, M.D., and James M. Anders, M.D., both of Philadelphia, Vice-Presidents; E. B. Gleason, M.D., Philadelphia, Treasurer; Joseph McFarland, M.D., Philadelphia, Secretary; John M. Swan, M.D., Philadelphia, Assistant Secretary; and John V. Shoemaker, M.D., Hobart A. Hare, M.D., Roland G. Curtin, M.D., Judson Daland, M.D., Orville Horwitz, M.D., all of Philadelphia, Councillors.

By-Laws

As adopted May 25, 1903, and amended June 5, 1917, and May 2, 1922.

Article I. The members of the Society shall be of three classes, Active, Corresponding, and Honorary.

Any regular American physician or scientist interested in the study of tropical diseases shall be eligible for Active membership.

Any regular physician or scientist not a resident of the United States, who has contributed to the knowledge of tropical diseases, shall be eligible for Corresponding membership.

Any scientist who has made eminent contributions to tropical medicine shall be eligible for Honorary membership.

The number of active members shall be limited to 300. Candidates for membership shall be elected until 1904 by the Council, and thereafter by ballot at the annual meeting upon recommendation by the Council.

Article II. The dues shall be fixed each year by the Council, but shall not be increased beyond $5.00 without approval by the Society at its annual meeting.

Active members shall pay the regular dues.

Corresponding members shall pay dues as determined by the Council but not exceeding the regular dues.

Honorary members shall be exempt from dues.

Article III. The Officers shall consist of a President, two Vice-Presidents, a Secretary, an Assistant Secretary, a Treasurer, and a Council of five members. They shall perform the customary duties of such officers.

The Council shall have general supervision of the affairs of the Society, and shall meet from time to time as they determine. The officers shall be ex-officio members of the Council. In 1917 one Councillor shall be elected to serve one year and one to serve two years. In 1918 one Councillor shall be elected to serve three years, one to serve four years and one to serve five years. Thereafter each Councillor shall be elected to serve five years.

In case of death or resignation of any officer or Councillor of the Society, the Council shall be empowered to fill such vacancy until the next annual meeting.

Article IV. There shall be an annual meeting and such other scientific or business meetings as the Council shall determine.

The annual meeting shall be known as the business meeting when the election of officers for the ensuing year shall be held, and routine and other business transacted.

Article V. These By-Laws may be amended by a two-thirds vote of the Society at its annual meeting as suggested by Council, provided such amendment has been offered to the Council one month previously, and recommended by it. Due notice of such contemplated changes to be sent out with the announcement of the annual meeting.

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Elected at the nineteenth annual meeting, June 25, 1923

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Stockman, Ralph..........................The University, Glasgow, Scotland.

MINUTES OF THE NINETEENTH ANNUAL MEETING

The nineteenth annual meeting of the American Society of Tropical Medicine was called to order at 9:15 a.m., Monday, June 25, 1923, at the Civic Auditorium, San Francisco, California, with President George Dock in the Chair. The first session of the meeting was attended by twenty members and visitors.

The Presidential Address was delivered by Dr. George Dock.

Dr. John V. Barrow presented a paper on "A clinical study of the intestinal protozoa, based on seven hundred and fifty cases." This paper was discussed by Drs. Kessel, Lynch, Ransom, Levy, and Dock.

Dr. Kenneth M. Lynch presented a paper on "Ingestion of red blood corpuscles by an intestinal amoeba with eight-nucleated cyst." This paper was discussed by Drs. Barrow and Kessel.

At 11:45 a.m. a business session was held during which the reports of the Secretary and Treasurer, and the minutes of the Council meeting including the Editor's report, were read and approved, the various recommendations as contained in the minutes of the meeting of June 24 were adopted, and the ballot of the Society was cast electing the 30 new active members, 5 new corresponding members, and the officers, councillor, and editor recommended by the Council for election to membership and various offices, respectively, as shown in the minutes of the said Council meeting.

The second session for the reading of papers was called to order at 9:10 a.m. Tuesday, June 26, 1923, at the Civic Auditorium, San Francisco, with Dr. Sidney K. Simon in the chair. This session was attended by twenty-two members and visitors.

Dr. Sidney K. Simon presented a paper on "Duodenal intubation as a means of detecting and treating parasitic infestations of the upper intestinal tract; with special reference to *Giardia intestinalis* and *Strongyloides stercoralis*." This paper was discussed by Drs. Walker, Ransom, and Lynch.

Dr. E. L. Walker presented a paper on "Contributions to the bacteriology of leprosy. II. The chromogenic acid-fast bacillus of Clegg." This paper was discussed by Dr. Lynch.

Dr. Walter V. Brem presented a paper on "Studies of malaria in Panama. V. Pernicious Malaria." This paper was discussed by Drs. Graves and Levy.

A paper by Dr. J. H. Chambers on "The complement fixation test and other findings in malaria" was read in abstract by the Secretary.

Drs. C. A. Kofoid and Olive Swezy presented a paper on "Pentatrichomoniasis in man." This paper was discussed by Drs. Walker, Lynch, Simon and Barrow.

Dr. N. E. Wayson presented a paper on "An investigation to determine whether clonorchiasis may be disseminated on the Pacific Slope." This paper was discussed by Drs. Kofoid and Ransom.

Papers by Dr. George C. Shattuck on "Results of treatment for clonorchiasis," Maj. Henry J. Nichols on "Graphic analysis of possible factors in involvement of the central nervous system in yaws and syphilis," Dr. Bowman C. Crowell on "The acute form of American trypanosomiasis; notes on its pathology, with autopsy report and observations on trypanosomiasis cruzi in animals," Dr. K. F. Maxcy and Dr. Leon C. Havens on "A series of cases giving a positive Weil-Felix reaction," and Dr. M. C. Hall and Dr. J. E. Shillinger on "The effect of magnesium sulphate, simultaneously administered, on the efficacy and safety of carbon tetrachlorid for the removal of hookworms" were read by title.

The Society adjourned at 12:15 p.m.

B. H. RANSOM,
Secretary.

REPORT OF THE SECRETARY, JUNE 24, 1923

There are no unusual activities of the Secretary's office to be reported.

Since the last meeting of the Society news has been received of the death of a distinguished Honorary Member, Prof. A. Laveran, May 18, 1922. The only other death loss in the Society's membership, since the last meeting, of which the Secretary has been informed is that of Dr. Guthrie McConnell, an Active Member, who died January 5, 1923.

The membership lists now stand as follows:

Active members	163
Corresponding members	18
Foreign honorary members	17

In addition there have been recommended by the Membership Committee for election at the 1923 Meeting the following:

Active members	30
Corresponding members	5

At the time of the report last year the lists stood as follows:

Active members	110
Corresponding members	11
Honorary members	29

Following the 1923 meeting the Society will still have over 100 members less than the limit of 300 provided for the active membership list by the By-Laws. Members are urged to recruit this list up to the limit so far as the supply of eligible material will permit. The continued welfare of the Society, particularly with reference to the publication of the official Journal, demands the support of a substantial active membership.

There are the following officers to be elected at the 1923 meeting: President, two Vice-Presidents, Secretary, Treasurer, Assistant Secretary, Editor, and Councillor for a term of five years, the term of Dr. G. C. Shattuck as Councillor expiring this year.

A delegate and alternate to the Executive Committee of the Congress of American Physicians and Surgeons for 1925 should be selected.

The time and place of the 1924 meeting of the Society should be determined.

B. H. Ransom,
Secretary.

TREASURER'S STATEMENT

Receipts

Balance, May 2, 1922	$564.71
Membership dues	971.39
From International Society of Tropical Medicine (Refund of membership fee plus accrued interest)	5.95
Library fund including accrued interest	34.65
Sale of Transactions	12.14
Journal subscription	5.00
From B. H. Ransom (error in bank deposit)	9.00
Total receipts, May 3, 1922 to June 23, 1923, inclusive	$1602.84

Expenditures

To Williams & Wilkins Co. for Journal subscriptions	$1219.00
Printing and stationery, offices of Editor and Secretary	52.00
Printing Transactions	114.75
Clerical and stenographic work, Secretary's office	20.00
Postage, Secretary's office	20.00
P. O. Box rent, Secretary's office	8.00
Stencil impressions, Editor's office	1.90
Rubber stamp, Secretary's office	1.05
Printing programs, 1923	11.00
Cut for illustration, Editor's office	14.94
Total expenditures, May 3, 1922 to June 23, 1923, inclusive	$1473.83
Balance, June 23, 1923	$129.01

B. H. Ransom,
Treasurer.

Examined June 25, 1923 and found correct.

E. L. Walker,
M. D. Levy,
Auditing Committee.

MINUTES OF THE COUNCIL MEETING

The Council met at 8:00 p.m. at the Hotel Whitcomb with the following present: Doctors Dock, (Chairman), Lynch and Ransom (Secretary).

It was voted to discontinue separate publication of the Transactions of the Society, inasmuch as they will appear in complete form in the American Journal of Tropical Medicine.

The report of Maj. Nichols, appointed a committee of one at the last meeting to investigate the question of the transfer of the archives and other property of the Society to a new location, was read and approved. This involves the placing of this property in the Library of the Office of the Surgeon General of the Army where it will be kept by the Librarian, with the probability that shelf space in the present library can be provided so that books, periodicals etc. in the Society's collection may be available for use. When the Surgeon General's Library is moved to the Walter Reed site there will be a small room available for this purpose. It will probably be called a study or research-room on tropical medicine and can house the archives of the Society indefinitely. The Council expressed its high appreciation of the courtesy and coöperation of the Librarian of the Surgeon General's Office, General Noble, in furthering this plan of establishing a place of deposit for the archives of the Society.

The resignations of Drs. James M. Anders, Lewellys F. Barker, Seale Harris, and F. F. Russell, active members, were accepted.

The following were recommended to the Society for election as active members: John V. Barrow (Los Angeles, Calif.), Nathaniel Bercovitz (Kachek, Hainan, China), Roger Brooke (Ancon, C. Z.), Bowman C. Crowell (Charleston, S. C.), E. D. Crutchfield (Galveston, Tex.), W. E. Deeks (United Fruit Co.), L. H. Dunn (U. S. P. H. S.), W. E. Eaton (U. S. N.), E. W. Goodpasture (Pittsburgh, Pa.), Brooks C. Grant (U. S. A.), Herbert Gunn (San Francisco, Calif.), L. W. Hackett (International Health Board), Mary J. Hogue (Greensboro, N. C.), Jas. E. Houghton (U. S. N.), Lee S. Huizenga (Taichow, China), S. M. Lambert (International Health Board), A. G. Love (U. S. A.), R. R. Parker (U. S. P. H. S.), Arlington Pond (Cebu, P. I.), Louis Schapiro (International Health Board), Geo. K. Strode (International Health Board), H. W. Stunkard (N. Y. University), C. E. Terry (Committee on Drug Addiction), A. M. Walcott (British Guiana), L. F. Warren (Brooklyn, N. Y.), Robt. Wilson, Jr. (Charleston, S. C.), N. M. Saleeby (Manila, P. I.), H. H. Steinmetz (del Carmen, P. I.), F. W. Vincent (Manila, P. I.), H. E. Stafford (Pasay, P. I.).

The following were recommended to the Society for election as Corresponding Members: Sebastiao M. Barroso (Rio de Janeiro), A. Pena Chavarria (Colombia), Solon Núnez (Costa Rica), Tomás G. Perrin (Mexico), Carlos Duran (Costa Rica).

The Editor's report, Secretary's report, and Treasurer's report were read and accepted.

An allowance of $25.00 per year for clerical and stenographic expense, and in addition remission of membership dues, was made to the Editor of the official JOURNAL, and a similar allowance and remission of dues to the Secretary-Treasurer, the allowances to begin with the year 1923 and the remission of dues to begin with the year 1921, said allowances to be paid out of unexpended funds in the Treasury.

A fund to be known as the Publication Fund was established, and an appropriation of $25.00 from the Treasury was made to this fund. The purpose of this fund is eventually to provide for more illustrations, more pages and other improvements in the Official JOURNAL.

Appreciation was expressed for the continued support of the AMERICAN JOURNAL OF TROPICAL MEDICINE by its publishers Messrs. Williams & Wilkins.

The Secretary was authorized to communicate to the members of the Society the substance of a letter received from Dr. W. S. Thayer relative to American Medical Aid for Russia.

In view of the fact that to function efficiently the JOURNAL needs a certain number of subscriptions of which not over half has yet been secured the Council approved a plan directed toward securing a greater number of subscriptions.

The Council recommended that as soon as the undertaking becomes feasible comprehensive abstracts, preferably in the form of critical and topical summaries of current papers on various subjects in the field of tropical medicine, be published in the Official JOURNAL.

The membership committee (Nichols, Butler and Ransom) and the Gorgas Memorial Committee (Lambert (A.), Meyer, Elliott, Thayer and Shattuck) were continued.

The membership dues for 1924 were fixed at the same rate as for 1923.

Dr. George Dock was selected as Delegate to the Executive Committee of the Congress of American Physicians and Surgeons, with Dr. John M. Swan as alternate.

The following were recommended to the Society for election as officers, councillors, and editor, respectively:

President, ALLEN J. SMITH
1st Vice-President, JOSEPH F. SILER
2nd Vice-president, GEORGE C. SHATTUCK
Secretary, BRAYTON H. RANSOM
Assistant Secretary, DAMASO RIVAS
Treasurer, BRAYTON H. RANSOM
Councillor for 5 years, SIDNEY K. SIMON
Editor, HENRY J. NICHOLS

It was voted to hold the annual meeting in 1924 at Chicago during the week of the meeting of the American Medical Association.

B. H. RANSOM,
Secretary.

REPORT TO THE COUNCIL ON THE JOURNAL, JUNE, 1923

The JOURNAL is now in its third year and is becoming well established professionally. The editor's aim has been to make the JOURNAL truly representative of the American spirit in Tropical Medicine and to avoid monopoly by any clique or specialty. It has been supported by most of the established agencies in Tropical Medicine and has also received welcome contributions from unexpected sources. In brief, on the scientific and professional side, with the papers from the Annual Meeting and from other increasing sources, there is no doubt that the JOURNAL can be maintained as a valuable medium of disseminating scientific medicine in the Tropics. Its good standard can even be raised.

Financially, as indicated by the inclosed report from the publisher, the outlook is not so good, but it is improving and eventually, of course, we must be self-supporting. Evidently we should continue our efforts to increase the subscription list by increasing our membership with suitable members and also by securing subscriptions from libraries and other institutions whose support will continue automatically.

As a personal matter, owing to a change of station, the editorial work for the last year has been done at the Second Corps Area Laboratory, 39 Whitehall St., New York, instead of in Washington where it more properly belongs. The work here has been somewhat handicapped by lack of clerical assistance, but the essentials have been carried on and it is expected that the work will be resumed at the Army Medical School this fall under more favorable conditions. Eventually, there should be some allowance for clerical work of the editor and also for that of the Secretary-Treasurer.

The Transactions of the Annual Meetings have been reprinted from the JOURNAL for three years in order to conform technically to the requirements of the Congress of American Physicians and Surgeons for the publication of Proceedings. This plan makes some trouble and expense for the Society and it is believed that, by any reasonable interpretation, the publication of the proceedings of the Annual Meeting in its own Journal is sufficient. It is recommended that separate reprinting of the transactions be discontinued.

Especial thanks are due to Captain F. Rodriquez, Dental Corps, United States Army, for the Spanish summaries which accompany each article.

The question of editorials, abstracts, and personal notes has been considered, but at present it does not seem feasible to start them. In general, it is recommended that the affairs of the Journal be allowed to go along in statuo quo for another year, when a further report will be made.

Henry J. Nichols.

American Journal of Tropical Medicine, July 1 to December 31, 1922, Covering Vol. II, No. 4 to Vol. II, No. 5

Incomes

Unearned Subscriptions		$903.42	
Future Volume Receipts		10.50	
		$913.92	
Subscription Receipts	$854.79		
Reprint Sales	244.44		
Advertising Receipts	55.10	1154.33	
		$2068.25	
Accounts Receivable December 31, 1922		485.50	
		$2553.75	
Less Accounts Receivable July 1, 1922		388.51	$2165.24
Future Volume Receipts	$677.50		
Unearned Subscriptions	63.78		741.28
Earned Income			$1423.96

Costs

Manufacturing Costs	$1189.63	
Distribution Costs	36.99	
Subscription Costs	447.66	
Mail List Costs	2.33	
	$1676.61	
General Expenses, Salaries, Rent, etc	380.71	$2057.32
Net Loss		$ 633.36

THE CULION LEPER COLONY[1]

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INTRODUCTION

The Culion Leper Colony is an interesting institution, and in many respects unique. For the study of leprosy and its treatment it offers most unusual opportunities. The largest well organized leprosarium in the world, it contains in large numbers all types of the disease and all degrees of advancement, except, of course, the earliest. The inmates are fairly amenable to reason, and little difficulty is experienced in obtaining adequate clinical material for any work desired. In spite of these facts the colony has not in the seventeen years of its existence become widely known, and it is seldom referred to when leprosy work is discussed. This is undoubtedly due to the fact that until very recently it has served chiefly as an isolation colony. Personnel and facilities have heretofore been insufficient to apply extensively, even on a routine basis, such anti-leprosy treatment as has been available.

With the access of interest in the treatment of this disease resulting from the reports of Rogers and co-workers from India, and, subsequently, those of Hollman and Dean and other Hawaiian workers, the desirability of extending modern treatment to the Culion inmates, and of utilizing the material for study was brought to the realization of the authorities controlling appropriations, and in 1921 provision was secured for starting such work on a small scale. The very keen and active interest of Governor-General Wood resulted, early in 1922, a few months after his inauguration, in the expansion of this work to a more nearly adequate scope. The knowledge of leprosy from the viewpoint of modern medical science is so inexact, and the problem of its treatment is so important and apparently still so far from solution, that it is out of the question that this work should fail to be productive of results of interest; it is to be expected that contributions will be made in more than one field. In view of this it has been felt that a description of the colony and of the work under way may be of interest in itself, and will help to orientate such reports as may be published in the future.

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EARLY LEPROSY WORK

The early history of leprosy in the Philippines is not of present importance. With Newberne,[2] whose interesting summary has been drawn on freely here, it seems unnecessary to attribute the appearance here of the disease to the act of the Emperor of Japan in sending, in 1633, 150 lepers to be cared for by the Catholic priests. The inhabitants of these islands have always had intercourse with the mainland of Asia, Japan, and other districts where the disease has existed since history began.

During the Spanish régimé some work was done by the ecclesiasticals for the special care of lepers. The first provision for them in Manila is said to have been a separate ward established in 1633 by Franciscan friars in their general hospital. A century and a half later, in 1784, Carlos III of Spain gave the San Lazaro estate, a district lying to the north of the walled city of Manila and across the Pasig river from it, to the Franciscans to establish a hospital for lepers. The greater part of the land was to be rented to raise funds for the support of the institution. This work was carried on until September, 1898, when San Lazaro Hospital was turned over to the American military authorities. There were two other leper hospitals established in the Philippines, one at Cebu, the dilapidated ruins of which were still used as a detention depot for the collection of lepers until recently ordered abandoned by Governor-General Wood, and one at Palestina, Nueva Caceres. In many of the larger towns there are said to have been groups of lepers more or less isolated, usually in old bamboo-and-nipa shacks. They were outcasts, without regular support or medical attention, and although they were accessible to friends the condition of many was extremely miserable.

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[2] Report of the Bureau of Health for the Philippine Islands, July to December 1913. Manila, 1914, p. 68 et seq.

The object of what regular work was done for the lepers during this period was humanitarian; it was not done for the purpose of eliminating the disease. The hospitals were primarily for the care of the needy cases rather than for segregation, and the inmates were apparently not strictly detained. It has been suggested that the very high leprosy incidence in Cebu may be due to the dissemination of the disease by many lepers, who had been brought there from other districts and who escaped and took up residence in nearby towns.

ESTABLISHMENT OF THE COLONY

It would appear that from the outset the American military and civil authorities alike were convinced of the desirability of instituting systematic segregation of lepers, but the establishment of the colony was not accomplished without difficulty. A military board investigated two islands, Culion and Cagayan de Jolo, as to their desirability for this purpose and decided on the latter, but the choice was so seriously criticized that a Committee of the Civil Government made an independent investigation late in 1901. Cagayan de Jolo was found entirely unsuitable because of the considerable population and poor water supply. On the other hand, Culion was found to be satisfactory. Plans were forthwith made to organize the colony centrally in the island, the approach to be by 3 miles of river and 2 of trail from a fine harbor that the investigators discovered in the southern portion of the island. After nearly a year of preparation on a small scale it was decided that this location was unsatisfactory on account of inadequate water supply, and the colony site was transferred to the little village of Culion, on the northeast coast. Construction work here did not commence until 1905. The delay, it is said, was due to conflicting opinions as to whether segregation would accomplish the extermination of the disease, and the difficulty of financing so large an undertaking. Late in May, 1906, the work had advanced sufficiently to permit the transfer of about 500 lepers, more than one-half of the maximum that was then being provided for.

The situation at Culion at the outset has been referred to as chaotic. This is not surprising. The staff was very small and it was difficult to obtain labor that was willing to work in the colony. There was one physician, Dr. Charles F. de Mey, who had been in charge of the preparation work, four French Sisters of Charity of the Order of St. Paul de Chartres, one Spanish priest, all on salary, and several other employees. The condition of the lepers when they came was extremely bad as a result of long neglect. Those not accommodated in the 100-bed hospital were housed in small bamboo-and-nipa huts for lack of dormitories, and their care was made difficult by inadequacy of most ordinary facilities. During the organization period the inmates undoubtedly had much to complain of, but this was to some extent unavoidable as a satisfactory system of management for such a place could not have been worked out beforehand. As the first, advanced cases disappeared and the colony increased it took on more of the aspect of an ordinary small Philippine town, with a number of concrete buildings added. The erection of these was spread over several years, the last being completed in 1916. After that there was little major construction until 1922.

THE PRESENT COLONY

The Culion Colony as it now exists is so large and the system so complicated that a full description is quite beyond the scope of this paper, but a brief statement of the general features is necessary if any understanding of the conditions under which the work is being carried on is to be had.

Culion Island, very irregular, meauring roughly 11½ miles in a north and south direction, and 19 miles in its longest diameter, with a total area of approximately 150 square miles, is one of the Calamian group, which constitutes the northern portion of the Province of Palawan. It lies about 200 miles to the south and west of Manila. With two other large islands and many small ones it surrounds a bay some 10 miles long, to which three deep-water passages lead.

The colony is on the bay side of the island and is everywhere surrounded by vistas of unusual beauty. But as one approaches by water, particularly if it be the sere dry season, one receives a most unfavorable first impression of a dreary, parched, poverty-stricken settlement on stony, unproductive hillsides. Actually the colony is for the most part a nipa-roofed Philippine town of 5000 inhabitants, more orderly, more cleanly, and, with its water system, electric lights, and ice plant, more advanced than most of similar size. However, a hasty survey during the hot part of the day is not apt to dispel the dismal first impression, particularly in those from northern regions, and opinions decidedly derogatory to the decision of the Commission that selected Culion for the colony have been heard. While it is true that there are distinctly unfavorable features, nevertheless Culion is not more unattractive than are many districts in the Philippines during the dry season. The beauties of the surrounding waterways and hilly little islands, rivaling the famed Inland Sea of Japan, the surprising response to treatment of the seemingly impossible soil, much of which is deteriorated soft stone fill, and the simple, orderly, not uncheerful lives of the inmates, greatly modify for the resident such first impressions.

The institution comprises the non-leper staff headquarters, Balala, and the colony proper, some 600 yards away. In neither of these districts, which together occupy but an insignificant bit of the island, is there any level ground that has not been made by cutting or filling. This character, though it makes development expensive and satisfactory ornamentation difficult, is distinctly fortunate from the viewpoint of town sanitation.

From the sea the colony begins as a rocky promontory, 30 meters high, crowned by a fine old Spanish church, the stone walls of which have withstood storms and Moro raids for over two hundred years. Below this is one of the treatment clinics with its nipa-roofed hospital, and several dormitories for children. Hospital Plaza, 18 meters above the sea, occupies the broadened saddle between this and the higher, 50-meter hill on and about which the greater part of the colony is built. This hill in turn drops back at one point to another, broader saddle, 30 meters above the sea, known as Worcester Plaza in honor of the Hon. Dean C. Worcester who, as Secretary of the Interior, played a leading part in the establishment of the colony. This saddle connects "Topside Hill" with the high L-shaped ridge that backs the colony; elsewhere the hill drops by flanking slopes to sea level. Balala is on the slope to the westward, a full ten minutes away from the center of the colony; the southern slope drops abruptly to a large amphitheatre-like hollow into which the town has extended until it has become necessary to move the old cemetery that was originally located there.

Farther to the south, separated from the colony proper by a kilometer of very steep hillside, is the Pilapilan district, a triangular flat deposited by the little mountain stream from which the colony water supply is obtained. There are here some 6 hectares (15 acres), of tillable soil, much of which the colonists are now cultivating. Across this flat to the east is the site selected for the proposed "negative colony" where cases that have become negative since coming to Culion can be segregated for the required two years. The less abrupt of the slopes that shut this flat in on all but the sea-ward side are fairly extensively planted, chiefly with bananas, pineapples and papayas. Some 2 or 3 miles west of Pilapilan is a much larger agricultural district, Baldad, much of which, unfortunately, cannot be cultivated with sufficient ease to be of use to the leper farmers, for as yet they have few farming tools and animals. Elsewhere the island offers a wide range of conditions, for open cogon-grass plains to dense tropical forests, from fertile river flats to stony little mountains. There is little spreading out by the colonists except along the coast; there are no roads and but a few foot-trails, and it is necessary for the colonists to go to the colony frequently for their ration issues.

The site originally selected for the colony was chosen with a view to encouraging agriculture, particularly cattle raising, but desirable as this would be it is doubted whether the colony would have developed along those lines. The majority of Filipinos are very gregarious and greatly prefer to live in close association when circumstances permit. At present less than 800 inmates live outside of the colony proper, carrying on agriculture, usually desultorily, or fishing; most of these are people from the Ilocos, Iloilo and Cebu provinces.

The total area covered by the colony proper is estimated to be about 40 acres, airplane view. On this there are over 600 houses of lepers and all of the government buildings; there is little waste space. Drainage is provided for in some districts by concrete gutters and conduits, though much of the colony is not so provided. The various midden sheds about the place and a few of the buildings are connected with tile sewers, most though not all of which empty into septic tanks. The water supply is from a small mountain stream, some 2 kilometers away. A 4-inch pipe line about a mile and a half long discharges into a large concrete tank from which most of the colony is supplied with running water; the districts higher than 35 meters will be supplied, and pressure for fire-protection purposes obtained, if plans now under way do not miscarry.

The Government-owned buildings constitute a considerable plant. On the filled flat of Balala is the Administration Building with, nearby, the wharf, a basin for small boats, store houses for supplies, a shed for the carpenters and mechanics, and the 3 small buildings and the radio tower of the Bureau of Posts. A trifle farther away is a general kitchen from which some 90 employees of various grades are fed, a mess hall, laundry sheds, a concrete barracks buildings now used for male nurses and others, and the house of the chaplains. A 4-apartment house for married physicians, and a bachelor's quarters for the unmarried ones, both of wood, and a long nipa-covered structure for the female nurses have been built for the new "treatment staff." On the hillside above are 7 residences, 3 recently erected, mostly occupied by senior members of the staff. A very cheap temporary building for hospitalizing non-leper laborers and others has also been placed on this level. The laboring force lives in a separate barrio some two-thirds of a mile to the west. The water supply of Balala is a very fine spring, high enough on the hillside to supply most of the flat by gravity.

The administrative center of the colony itself is Colony Hall, with the court-room of the Chief of the Colony above and the Government store and the colony post-office on the ground floor. Nearby is the General Kitchen, from which some 300 inmates now draw cooked rations and the rest obtain their daily fish or fish substitute, the weekly beef, and such vegetables as are available from time to time. Other structures include a distributing shed from which are issued the weekly dry rations, of the nature of groceries as compared to the provisions issued from the general kitchen; a headquarters shack for the various activities of the nature of public works, including among others plumbers, tinners, painters, carpenters, firemen, and street cleaners; an incinerator for such garbage as cannot be fed to the domestic animals of the inmates; a primitive jail and detention quarters for insane in one of the original Culion houses; a quarantine station for newcomers in two others. One building is devoted to the school, which is supported by the Bureau of Education; there are five grades, the highest of which the Philippine Anti-Leprosy Society materially helped to establish. Another building is the Leper Club, with a piano, a pool table, and many newspapers, some recent, and miscellaneous discarded "charity" magazines and books unintelligible except for the pictures to all but the comparatively educated few.

The various dormitories house a total of about 2000 persons. Some of these are concrete "tenement houses," simple 5-room affairs with 12 persons assigned to each room, which measures 5 by 7½ meters. Until recently there were 10 of these tenements, but 5 have been taken over by the medical section; 4 wooden barracks for 50 inmates each were built last year to permit this. Several other buildings are used as living quarters, the number of adults accomodated being about 1350. For children there are 10 dormitories of various kinds and sizes, in which 650 individuals are crowded. Two were built mainly by the Catholic authorities, and 2 small ones by the Mission to Lepers.

Though at the outset it was, naturally, expected that quarters would be provided for all of the inmates, this has never proved possible. Most of them (over 3000), are living in houses built and owned by themselves, or rented from other lepers. Most of these houses are small, made chiefly of bamboo and nipa; the values range, when new, from $25 to $60, though not a few are more pretentious.

For the medical work there was, previous to 1921, only the so-called General Hospital. Until very recently this consisted of 6 wards with 300 beds crowded into them, a kitchen, and a small morgue. At present, after some rearrangements, there are 7 wards with a total capacity of 265, crowding having been relieved to a considerable extent. A new kitchen has been built and the old one is to be the colony pharmacy. In the oldest of the hospital buildings are 2 wards, and rooms used by the Sisters of Charity and the medical staff. The old colony pharmacy, still accommodated here, is to be converted into an operating room, there having been heretofore no special provision whatever for surgical work. Under one wing of another large concrete building which contains 2 wards are several rooms, in which are quartered an ulcer-dressing clinic conducted by one of the two Sisters who have been at Culion since the first patient arrived, a medical clinic about to be developed, a class-room for the leper nursing aids, and a medical supply depot from which the wards are now furnished the minor supplies needed in their daily work. A fifth ward is in a small separate concrete building which, it is expected, will be used as a radio-therapy department as soon as the necessary equipment arrives. One ward, 30 by 8 meters, has been taken over for a laboratory and office building; the patients have been provided for by taking over 2 buildings previously used as tenements. The morgue building has a usable autopsy room, but is without provision for refrigeration, a very real handicap in pathological work in this climate.

Antileprosy treatment is given independently of the hospital. When the new work was begun in 1921 a 6-room concrete building that had been erected for the segregation of negative lepers was taken over, and a wood-and-nipa clinic "emergency" hospital of 50 beds capacity was erected near it for the care of lepers under treatment suffering from incidental illness. After the staff was finally enlarged in 1922 a 5-room concrete tenement building in another district of the colony was taken over for the second clinic center. Its hospital, of better material and different design than the first, has been built near it; the capacity is 50 beds and the cost was approximately $3000.

The low Pilapilan district being a focus of malarial infection, a small cheap clinic was erected there last year to facilitate combating that infection, as well as to save the residents the trouble of going to the colony for treatment. The residents of the Baldad district have now petitioned for a similar clinic there, which it is expected can be provided shortly.

The administrative center of the treatment work is in the old Ward 6, in which the offices of the Chief Physician and Chief Nurse, and the headquarters of the pathological and chemical sections have been installed. This close association has proved very advantageous in the few weeks that the building has been occupied.

Until February of the present year the pathological section was quartered in a single large room in one of the clinics. This had been arranged in 1921 when there was only the simplest equipment and a single laboratory man doing part-time clinical work. In the newly arranged quarters there is provision for 4 or 5 workers in addition to a room for technical assistants, a preparation room, a small examining room, and small but adequate dark rooms. The equipment is in most respects adequate for the work planned.

The chemical research laboratory is in the same building. This is equipped for such work as has been anticipated will be needed for our purposes. The manufacturing chemical plant is entirely separate, and has been in operation since September, 1922. At capacity some 100 liters of chaulmoogra ethyl esters or equivalent products can be produced a month.

GENERAL ORGANIZATION

The entire Culion reservation, which includes several nearby islets, is the property of the Philippine Health Service, and is strictly reserved; even the semi-wild pagans (Tagbanuas) of the region realize that landing here is not permitted. The Chief of the Colony, as the representative of the Director of Health, is in full control, functioning as general administrator, Justice of the Peace, and counselor of the inmates. Until recently his responsibilities included the medical service, and some of the incumbents, particularly Dr. O. E. Denney, now in charge of the Federal leprosarium in Louisiana, have paid much attention to this side of the work. But as the institution grew it became necessary to pay more and more attention to other phases, and with the final increase of staff in 1922 the fields of operation were, by mutual agreement, divided. The Chief of the Colony now directs the sanitary work, in addition to the numerous other general administrative activities; the Chief Physician is responsible for the work of the medical section and the professional performance of its personnel, which includes, besides the clinical staff, the nursing division, the pharmacist, and the dentist; and the Chief Pathologist and the Chief Chemist are similarly in charge of their fields. It is expected that, on the appointment of a permanent Chief Physician, the four heads of sections will act together as a Board in dealing with general problems and fixing general policies.

The non-leper staff of the administrative section consists of a few permanent (civil service) employees, two Catholic priests, assisted by a Lay Brother, and a considerable but variable number of temporary employees, including those of the office, store-room, kitchen and bakery, the shop and construction forces, sanitary personnel, and miscellaneous labor.

In the colony practically all of what may be termed routine work is done by leper employees. Aside from the medical and technical staffs, the carpenters and most of the laborers on special construction projects, and the *cargadors* who carry supplies from the Balala store-house to the colony, there are no "sano" employees. Order is maintained by a well disciplined corps of leper police, under an ex-constabulary soldier. A corps of "bomberos" is drilled in simple fire-fighting tactics which, fortunately, have been needed but once since the force was organized.

The staffs of the General Kitchen, the Government store, the colony branch of the post-office, the Public Works department, the principal activities of which have already been indicated, the sanitary department, the court, the band and the orchestra, are all lepers. Most of the routine nursing and all of the service work of the hospitals and clinics is done by leper employees.

The government of this fair-sized town is carried on by the Chief of the Colony, in part through and with the aid of the Advisory Board, an elective body of which he is, *ex officio*, the head. The political division is not by districts within the colony but by the racial subgroups, as the Tagalogs, Cebuanos, Ilocanos, etc. The important ones have their own representatives chosen in biannual elections which are often warmly contested.

The various material needs of the colonists are provided in varying degrees of adequacy. Their spiritual welfare is largely in the hands of the two priests, and of the Sisters, all of whom are on salary. The Protestant inmates, who are greatly in the minority, are aided by a missionary and his wife, who now spend about one-half of their time at Culion. Their work is supported largely by the American Mission to Lepers. All of these render most valuable service particularly in the care of the children of the colony, and the high order of their devotion and self-sacrifice cannot be over-rated.

For the amusement of the inmates some little provision is made. Cinematograph shows, of very cheap films, are given irregularly, averaging perhaps twice a month. A brass band and a string orchestra composed entirely of lepers are paid monthly wages to play alternately on Sundays and Thursdays in the main plaza. Cock-fighting, a national sport, is permitted on Sundays and holidays, efforts being made by regulations to discourage unreasonably heavy betting. A few pool and billiard tables are operated privately, and some little home gambling is quietly indulged in about the place, usually on a very small scale. Occasionally games of indoor baseball are played, and a single tennis and volley-ball court has recently been equipped. On two occasions colonists have organized theatrical companies and have staged fairly successful performances. For the most part the inmates lead very quiet, uneventful lives, finding such entertainment as they may in very little things.

Of the food, the main staple is rice; the principal minor issues are mongo beans, lard compound, sugar, tea, coffee, garlic, onion, and canned tomato. All of these are brought from Manila and are issued weekly. Fresh fish, of which each inmate is entitled to 250 grams daily, has usually had to be substituted in some part by canned salmon or sardines, or by mongo beans; the amount of fish that can be used daily is 1300 kilos, whereas for 1922 the daily catch averaged but 400 kilos. Until late in February, 1923, this was gotten entirely by leper fishermen, who depended very largely on cheap traps on the coral reefs and mud flats, but recently a crew of Japanese fishermen, by very strenuous methods by which they meet the fish in their own element, have been obtaining sufficient quantities to fill the daily quota. Beef, of a very inferior quality produced on nearby islands, is issued once a week, though it is provided three times a week for hospital patients. Of vegetables there is a very unfortunate lack; because of high ultimate costs and irregular supply little more than onions, garlic and sweet potatoes are shipped from Manila. For the most part such products as can be purchased from leper farmers are depended upon. In 1922, at the suggestion of Miss Hartley Embrey, of the Pekin Union Medical School, mongo sprouts were regularly used at least twice a week, but the lepers tired of these after several months and their issue had to be discontinued. Steps were then taken to encourage the lepers to plant vegetables, either on their own premises or on a larger scale in the outskirts of the colony. A home vegetable garden contest was instituted with good results, though there still remains much to be done in this line.

The regular issues of clothing are decidedly inadequate. Only in 1920 and 1921 was it possible to issue clothing twice a year; the average annual allowance in previous years was $1.00 per inmate. The slight, irregular aid from charitable sources does not prevent this factor being one of the strongest incentives to self-help on the part of the inmates.

The regular "gratuity" money allowance of $0.10 a week to each leper provides little substantial help, though it is held very important by those who without it would never have even cigarette money. By mutual consent this is not paid to able-bodied men, and the fund so saved is used to pay some of the necessary colony workers who are not provided for by the regular appropriation. Among the remunerative occupations are the various Government-supported activities already indicated, and many private enterprises, including the keeping of *tiendas* (stores, for the most part extremely tiny), agriculture, fishing, wood-cutting and bamboo-working and many minor ones. There are two bakeries of some size, and an ice-cream restaurant. The only shoemakers at Culion are lepers and the staff depends on them entirely for such work.

It is most unfortunate that the Government cannot afford to house, feed, and clothe all of the inmates more adequately. To diminish the very high tuberculosis incidence there should be less congestion in the houses. For the same reason and also to aid the anti-leprosy treatment the food should be more abundant and varied. The young, the pregnant, the old and the weak obviously should have particular care, and to some extent this is done. The average daily ration cost is in the neighborhood of 12 cents gold, whereas at San Lazaro Hospital in Manila, where 200 to 300 cases are usually confined, it is twice as much. However, many of the Culion inmates have means of self-help, so the situation is not as bad as it would seem. As a matter of fact, were it possible financially to provide fully for all it would be very difficult to find enough workers to carry on the necessary colony activities. And for the majority it is believed that from the sociological and medical viewpoints some regular light occupation is desirable. It would undoubtedly be harmful for the average active leper, as for the average healthy human being, to have all his wants met without effort on his part. The additional cost of having all work done by non-leper employees, if, indeed, such could be obtained, would be considerable.

The care of lepers, including their collection, support and treatment constitutes a considerable item in the annual budget of the Government. For 1923 approximately $550,000 will be spent in this work, which is nearly 1.7 per cent of the estimated total income of the Government, $32,500,000. This amounts to practically $100 per leper, and to $0.055 per inhabitant of the Philippines. Though this is very small as compared to the expenditures in Hawaii, which are approximately $300 per leper, and $1.00 per inhabitant, nevertheless it is a more serious drain than would appear from these figures, on account of the low per capita wealth in the Philippines, the comparatively undeveloped economic state of the country, and the many expensive activities that the Government has to maintain.

The Philippine Anti-Leprosy Society was organized late in 1921, with the original object of raising funds to aid the Government to provide the lepers better treatment and care, but except for the purchase of steam laundry machinery, and a small motion picture machine for use in hospitals and dormitories, it has been able to do little as yet.

The local lodge of the Knights of Pythias has shown much interest in the work of late. Captain G. W. Spoerry, on discovering that a member of the Manila lodge is an inmate of Culion, started a campaign for an appropriation of $1,000,000 from the Grand Council, chiefly to build, equip and endow adequately a research laboratory. A local drive netted enough money to establish a small electrically equipped tailor shop, for which no operating personnel has as yet been provided. No action was taken by the Grand Council on the endowment proposal, though the Associated Press sent out a dispatch to the effect that the appropriation had been made, which statement has been reprinted in medical and scientific journals.

It is very greatly to be hoped that some such endowment, or other outside aid to extend the work now under way may be forthcoming. Culion at the present time offers most favorable conditions for the study of leprosy and its treatment, but research cannot be put on a sufficiently broad basis from the Government exchequer alone.

TREATMENT WORK

So much for the general setting in which the treatment work is being carried on. There are various general problems aside from the medical work that are of interest, particularly in connection with the question of compulsory segregation of lepers, but these cannot be discussed here. Under the term "treatment work" as used here is included, besides the activities of the medical section, with its anti-leprosy treatment clinic and the general care of sick lepers, those of the pathological section, with its routine examinations, autopsies and special work, and of the chemical section, which manufactures all of the antileprotic preparations used, and is engaged in the study of new ones.

Most of the personnel of these sections is provided for by a special act of the legislature passed early in 1921, which limits the number of "medicos y tecnicos" to 20 in addition to the 3 physicians, not including the Chief of the Colony, already at Culion when the act was passed. All but 2 of the 20 positions are now filled. Two years ago there were 4 physicians at Culion, 1 of whom was the administrator. One of the others served also as pharmacist, so there were practically the equivalent of but 2½ men to care for 300 hospital patients, a daily "dispensary" clinic of 50 to 80 cases, and calls from outside the hospita . Thus undermanned, with no modern hospital equipment, it is not to be wondered at that there are on file no records of value. When, in 1921, the staff was increased the situation became even worse so far as the general work was concerned, as one of the old staff became largely engrossed in the new treatment work. Thanks to the recent increase there are at present 15 physicians in the medical section, including the Chief Physician. Two groups, comprising 2 supervising physicians and 9 treating physicians, are in charge of the treatment clinics, and 3 men are assigned to the General Hospital.

The nursing staff consists of 10 Sisters of Charity, who concern themselves solely with the General Hospital, and 20 graduate nurses of whom at present 11 are females and 9 males. It is impossible fully to evaluate the work of the Sisters who, since the colony was started, have worked with unflagging devotion under most monotonous and sometimes extremely trying circumstances. Under the Chief Nurse, there is in each clinic and attached hospital a supervising nurse and corps of nurses; a few are assigned to the General Hospital to assist the Sisters in technical matters, and one is attached to the Negative Children's Home and the infirmary in Balala. The nursing aid staff is an important factor. The leper ward attendants, heretofore permanently employed, without instruction, have so far as possible been put on the basis of students in an elementary training school, with regular classes, better food and quarters, and other advantages. They are paid according to rank, and promoted on passing examinations. Considerable improvement in service has already resulted.

A competent pharmacist has been added to the staff. He is assisted in the colony pharmacy by one of the Sisters, and so is able to fill the recently created office of Medical Supply Officer. A dentist has for some years kept an office in a somewhat inaccessible room, until recently without running water. A dental chair has now been acquired, though the instruments are as yet too few for satisfactory work. The dentist makes amalgam and cement fillings and treats local infections in patients who apply. Unfortunately, these are comparatively few, though the mouths of many of the lepers are in very bad condition. The dental office will shortly be moved to better quarters and an educational campaign started.

The staff of the pathological section nominally consists of a Chief Pathologist and 2 assistants, with 3 technical assistants of various degrees of training; the present Chief Pathologist has found it impossible to devote time to this work on account of other, temporary duties. Aside from routine clinical laboratory examinations, autopsy work is the most important present activity of the section. No laboratory studies could be carried on in the crowded quarters until very recently occupied, and no extensive program can be undertaken until a permanent Chief Physician is appointed.

The staff of the chemical section, under Dr. Granville A. Perkins, formerly of the Bureau of Science, Manila, includes 2 assistant chemists, who are graduate pharmacists, and 2 semiskilled helpers. This section manufactures all of the preparations used for antileprosy treatment, chiefly the ethyl esters of chaulmoogra oil, plain and iodized, and on a small scale the ethyl esters of cod liver oil and the Mercado mixture; the Rogers solutions of sodium salts were discontinued after several months use in 1921. Dr. Perkins is endeavoring to prepare materials that will be more active, or less harmful, than those now in use. This section will be depended on largely for special blood and urine chemical work so long as the pathological laboratory force remains as small as at present.

A word of appreciation of the spirit with which the staff members have carried on the work is in order. The conditions of life in this isolated, none too well endowed institution are to say the least not the most desirable, and many are here at a distinct financial and social sacrifice, yet as a whole the staff is devoting itself, cheerfully and interestedly, to benefit the cases under their care and advance the knowledge of leprosy and its treatment.

As has been said, anti-leprosy treatment is given in 2 larger and 1 small treatment clinics. Each of the 9 clinic units is manned by a physician, a nurse, and 2 leper nursing aids. An average of nearly 500 cases is registered in each of these units, the total registration being practically 4500. Every suitable case is supposed to be injected once a week, and in a few small groups twice. Actually, the injections given each week average about 60 per cent of the total registration. Many cases have to be excused on account of some disturbance or other, and more absent themselves without valid reason. Preparations are now being made to compel attendance by holding up part of the rations of absentees until they have reported to their physicians.

Until last year a sort of general dispensary clinic was maintained at the General Hospital where those not registered under a physician could report for treatment of incidental and concurrent conditions. As the chief responsibility of the staff at that time was to get as many cases under treatment as possible, this clinic was closed to induce registration in the treatment clinics. Among the cases so registered were many who, on account of pulmonary tuberculosis, nephritis or other conditions are considered not suitable for anti-leprosy treatment. Therefore, each unit physician has had a "dispensary" group supposed to report regularly for observation and such treatment as might be indicated.

Patients of a clinic, whether treatment or dispensary cases, who required temporary hospitalization for any cause are placed in the clinic hospitals. One of the wards of the General Hospital has been taken over for the women of all clinics pending the erection of a separate building for them. Cases that become permanently bed-ridden are transferred to the General Hospital service.

The General Hospital proper is reserved for cases that are unsuitable for clinic treatment and require hospital care. Not a few of these are in good health but require nursing attention, being unable to do for themselves on account of ravages of neural lesions. The equipment has been almost primitively simple. Surgical and other instruments, scales, and blood pressure, aspiration and infusion apparatus have now been procured, and additional equipment, including X-ray, quartz light and electrotherapeutic apparatus, from which much benefit is expected, is being added this year.

After a year of reorganization and preliminary observation certain changes are being made in the system as outlined. The clinic registration cannot well be increased without more staff, and this has not been allowed, yet new, favorable cases are being brought to Culion and are appealing for treatment. Furthermore, since the present available methods are undoubtedly far from the best possible, it is imperative that clinical and laboratory research be pushed. To meet these conditions the senior physician of the General Hospital has been relieved of all but supervisory duties there and is serving as Assistant to the Chief Physician, on special assignments. One of the clinic physicians has been relieved of routine treatment work, and another may be relieved later. All clinic cases who are unsuitable for treatment will be transfered from the clinic register to an organized dispensary clinic, and the suitable cases redistributed and as many of the recent arrivals taken on as possible. The dispensary clinic will be at the General Hospital, under 2 men, which will insure more uniform treatment. Work on tuberculosis, nephritis and other complications of leprosy can be centered here. Research is not being confined to this team, however, and every member of the staff is encouraged to make special observations on some phase of the work.

Nothing can be said here concerning the results of the work done so far, aside from the general statement that they are distinctly encouraging, though far from being as spectacular as those reported from Hawaii.

Not the least encouraging feature of the leprosy situation is the fact that for the first time since active segregation was inaugurated, cases are voluntarily reporting to the authorities for treatment. Heretofore they avoided apprehension since segregation, though not absolute in that visitors are permitted at Culion, was practically so on account of the inaccessibility of the colony.

It will be seen that, in spite of many deficiencies remaining, this colony now offers material and facilities for the intensive study of leprosy and its treatment on a modern basis that is without parallel. Even without further improvements in the matter of personnel, and of equipment other than those provided for the present year, much can be done. With the present establishment as a basis, any additional support will be productive of results greatly out of proportion to the investment.

SUMARIO

La Colonia de Leprosos de Culión, en las Islas Filipinas, es hoy la mayor leprosería del mundo. A pesar del hecho de que ya han transcurrido unos diecisiete años desde su fundación,

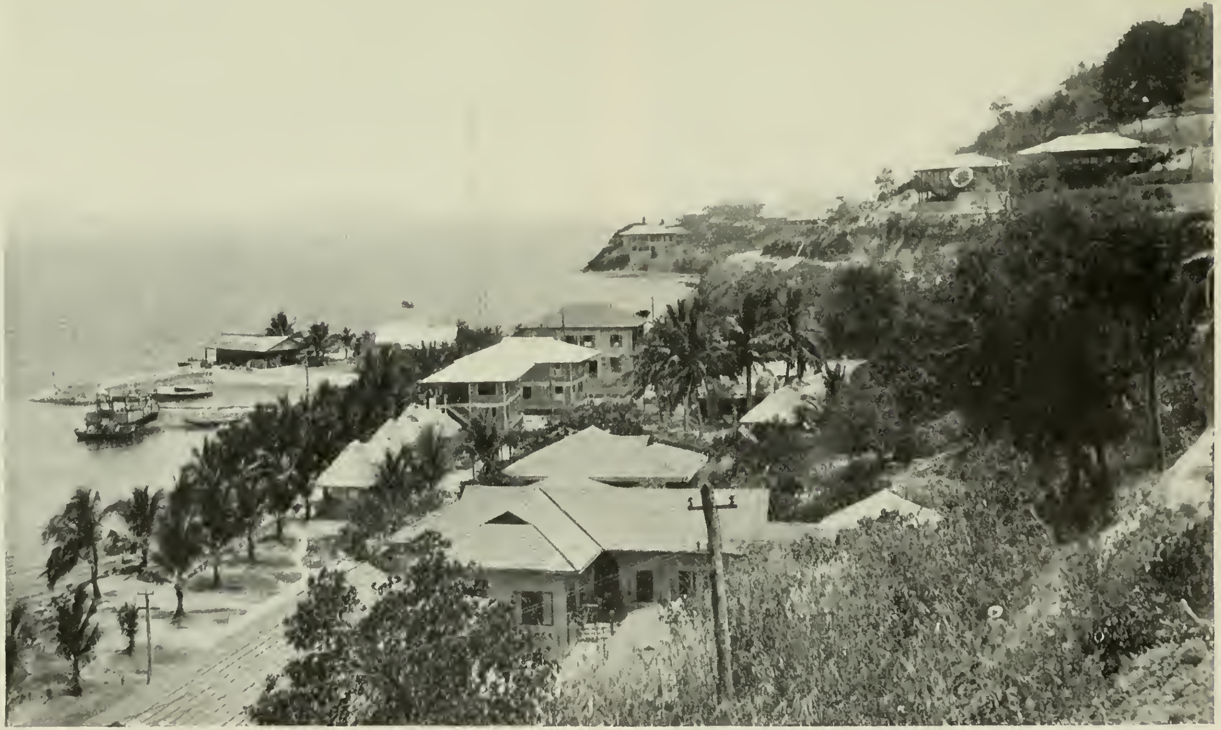
llama la atención el que no se le mencione más a menudo en los escritos profesionales que tratan sobre el tratamiento colectivo de la lepra. Puede que esto se deba al hecho de que hasta hace poco tiempo la Colonia sirvió solamente de punto de aislamiento y a que tanto el personal como las facilidades disponibles eran insuficientes para atacar el problema en grande escala- Sin embargo, gracias en parte al interés personal que se tomó el Gobernador General Wood desde poco después de su inauguración, el gobierno votó un crédito en 1921 que ha permitido la expansión de este trabajo de una manera adecuada.

Durante el régimen español el cuidado de los leprosos estaba a cargo de los eclesiásticos. At terminarse la guerra hispanoamericana ya existía el Hospital de San Lázaro, hacia la parte norte de Manila, bajo la dirección de frailes Franciscanos y construído en terrenos cedidos para el propósito en 1874 por Carlos III de España. Habían también por entonces dos hospitales mas, uno en Cebú y el otro en Palestina, los cuales no servían mas que de puntos de congregación. En algunas de las poblaciones más grandes se habían reunido grupos de leprosos, más o menos aislados, quienes ademas de carecer de asistencia medica sobrellevaban una vida miserable.

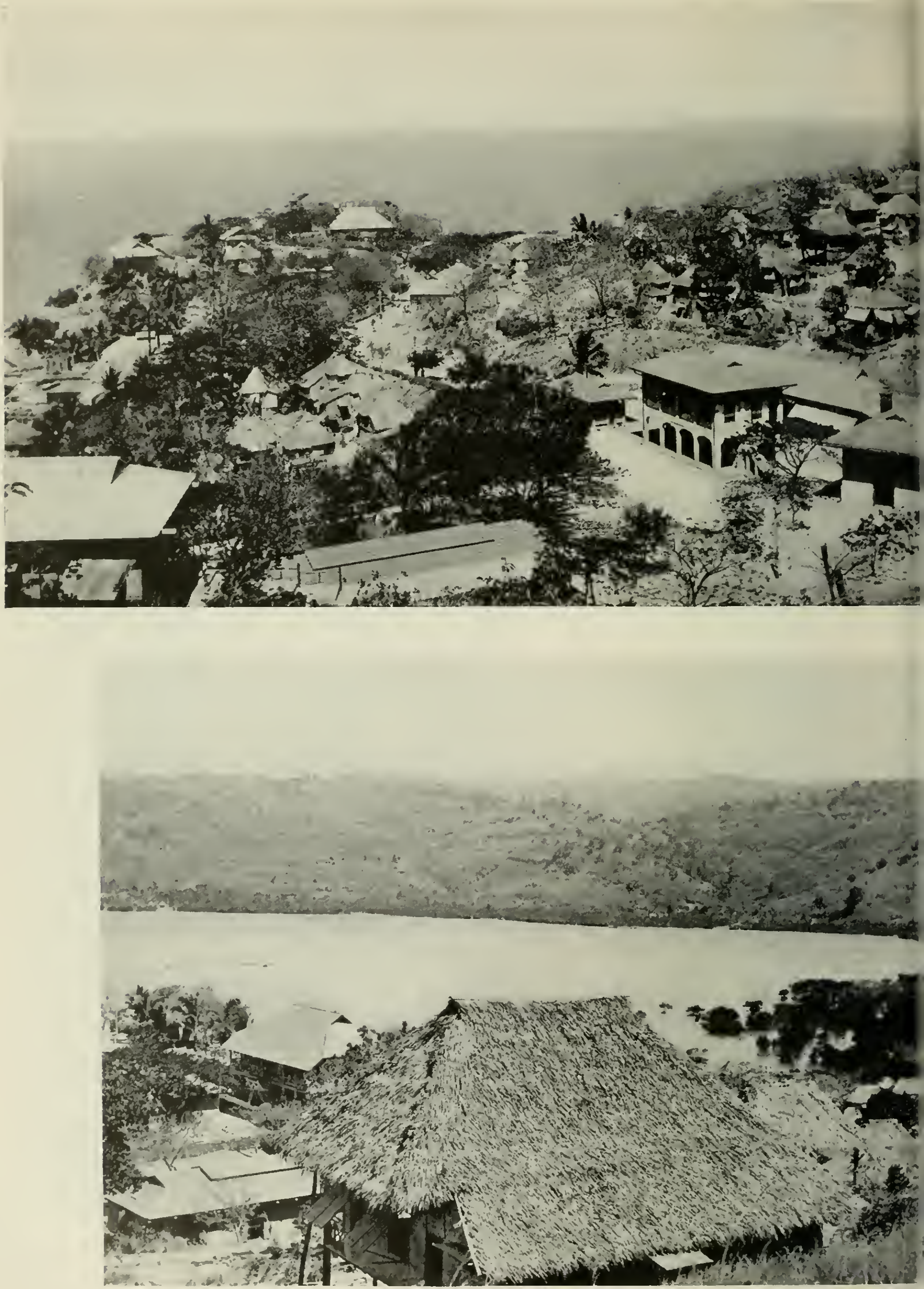
La segregación sistemática de leprosos empezó con el régimen militar americano, ha continuado con vigor desde entonces, y promete, tan pronto se hayan introducido todos los métodos y mejoras modernos, resultar de una gran utilidad pública y cientifica.

La Colonia está radicada hacia la parte noreste de la isla de Culión que se ha dedicado enteramente para este fín. La isla comprende unas 150 millas cuadradas de superficie y está situada hacia el norte y a poca distancia de la provincia de Palawán. La colonia propiamente dicha ocupa un área de cuarenta acres. Además de las casas de administración, residencias del cuerpo facultativo y ayudantes, hospital, escuelas, etc., se han construído 600 casas para viviendas de leprosos. En la reserva propia hay 1350 adultos y 650 menores acomodados, de los cuales 300 son casos de hospital. De todos los puntos de la isla hay 4500 individuos registrados. El cuerpo facultativo lo componen 15 médicos, 3 técnicos, 20 enfermeros de ambos sexos y 10 hermanas de la Caridad. Todo el trabajo de rutina lo hacen empleados leprosos. En la actualidad el presupuesto anual para el mantenimiento de la institución es de 550,000 dólares americanos.

Tiene departamentos de Patología y de Química cada uno de ellos a cargo de un director y cierto número de ayudantes. La planta química tiene una capacidad productiva de 100 litros diarios de los éteres etílicos del aceite de chaulmogra. La planta fabrica también el éter etílico del aceite de hígado de bacalao y la preparación de Mercado. El uso de la solución de sodio de Rogers fue suspendido en el 1921. El tratamiento se lleva a cabo en tres clínicas especiales. Cada caso adecuado se inyecta una vez a la semana. Varios grupos pequeños de pacientes reciben dos inyecciones a la semana. Aunque los resultados hasta ahora obtenidos son en verdad alentadores, no existen razones para caracterizarlos con todo el sensacionalismo que emana del Hawaii.



Culion Leper Colony—A View of Balala, the Non-Leper Staff Headquarters
A bit of the colony proper is seen in the background



Culion Leper Colony—Panoramic View of Major Portion of the Colony F
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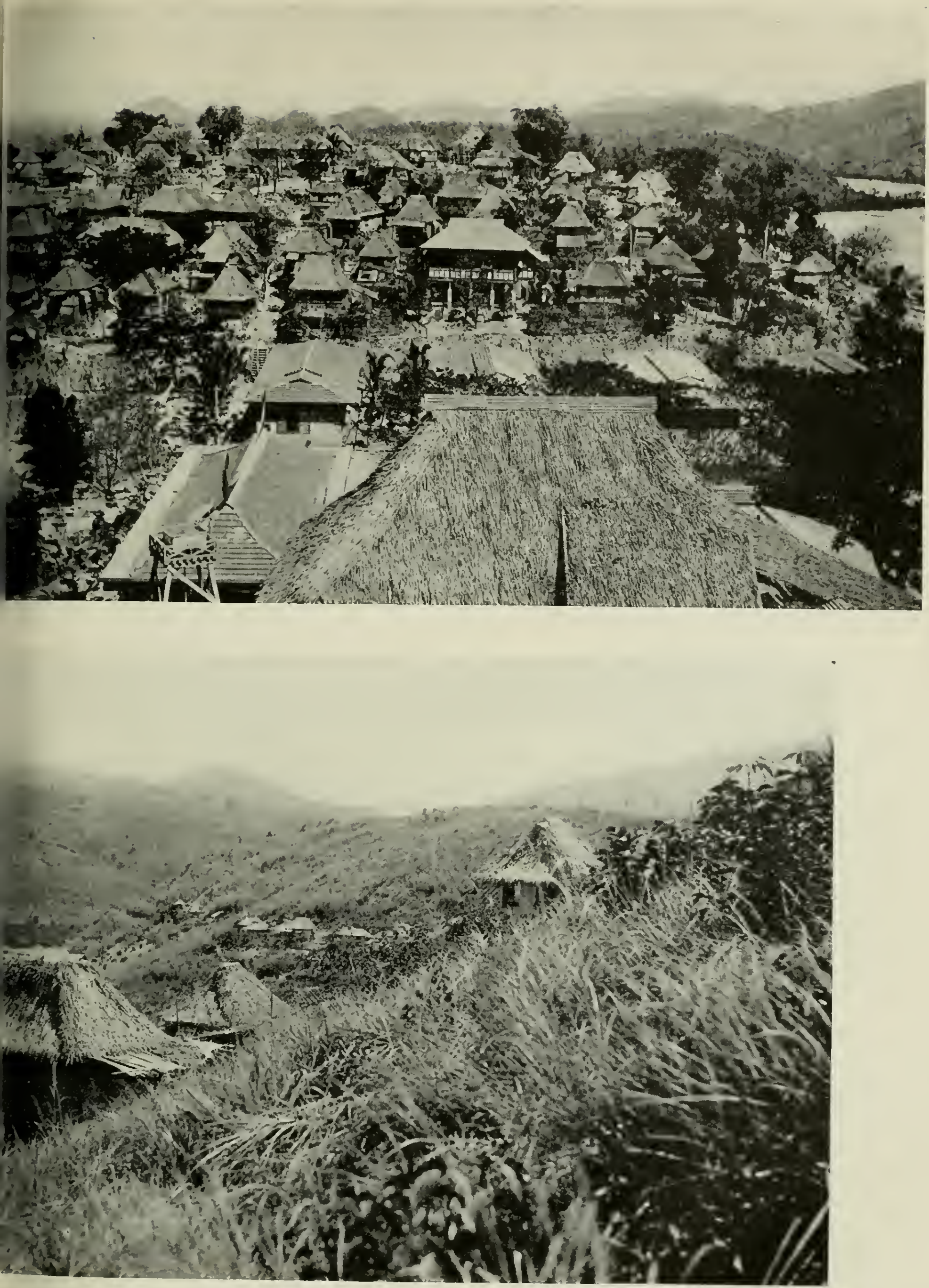

THE PRINCIPAL GOVERNMENT BUILDINGS AND MANY TYPICAL HOUSES OF THE COLO-
E ISLAND TO THE EAST AND SOUTH

CONTRIBUTIONS TO THE BACTERIOLOGY OF LEPROSY

II. THE CHROMOGENIC ACID-FAST BACILLUS OF CLEGG[1]

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In a previous paper (1) I have called attention to the apparently unique position held by the bacillus of leprosy. Although this bacterium has been known for some fifty years and is present in enormous numbers in leprous lesions, no one has succeeded in cultivating an organism from these lesions entirely comparable with the bacillus in the tissues. It was also pointed out that of the organisms, that have been cultivated, two, the partially acid-fast diphtheroid of Bordoni-Uffreduzzi and the chromogenic acid-fast bacillus of Clegg, have been isolated repeatedly from leprous lesions by competent bacteriologists working in different parts of the world. In that paper I discussed the cultivation and identity of the first of these organisms, the pleomorphic partially acid-fast diphtheroid. The more or less constant cultivability of this diphtheroid from leprous lesions was confirmed, and it was shown that this organism is either identical with or very closely related to a diphtheroid that is the only bacillary organism cultivable on ordinary media from smegma praeputii.

The present paper is concerned with the second of these bacterial organisms cultivable from leprous lesions, the chromogenic acid-fast bacillus of Clegg. In 1909 Clegg (2), at Manila,

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[1] Read at the nineteenth annual meeting of the American Society of Tropical Medicine, San Francisco, Cal., June 25 and 26, 1923.

Preliminary report. Owing to the transfer of the leper patients from the San Francisco Isolation Hospital to the National Leprosarium at Carville, Louisiana, this investigation has of necessity been discontinued for the present.

Philippine Islands, announced the cultivation of an acid-fast bacillus from leprous tissue in symbiosis with amoebae and cholera vibrios on the medium used by Musgrave and Clegg (3) for the cultivation of amoebae. This medium consists essentially of agar and water and is of very low nutrient value. Its purpose is to inhibit the growth of bacteria which, although necessary as food for the amoebae, must not overgrow them. In a later publication Clegg (4) reported the repeated cultivation of this acid-fast bacillus from leprous lesions by the same technic, and the isolation of the acid-fast organism from the symbiotic amoebae and cholera vibrios by heating the mixed culture to 60°C. and transplanting to ordinary nutrient media.

Clegg evidently considers the essential factor of his technic to be the symbiotic amoebae which provide a condition of growth for the lepra bacillus somewhat approaching the intracellular parasitism in the tissues. He calls attention to the difficulty of obtaining the initial growth of this acid-fast bacillus, in that of a large number of cultures made from the same material containing innumerable lepra bacilli only a few will show growth of the acid-fast organism. The surprising fact is that when growth of the acid-fast bacillus is once initiated in the symbiotic cultures, the bacilli may be isolated from the amoebae and will grow readily and rapidly on ordinary nutrient media. Clegg's bacillus is shorter and thicker than the lepra bacillus in the tissues, is facultative acid-fast and produces a yellow to orange colored pigment.

Clegg continued his investigations at the Leprosy Investigation Station of the United States Public Health Service in Hawaii, where he and his collaborators and successors, Currie, Brinckerhoff and Hollmann (5), Currie, Clegg and Hollmann (6) and McCoy (7), cultivated the chromogenic acid-fast bacilli repeatedly from different cases of leprosy and isolated the organism in pure culture by the technic of Clegg.

Duval (8) in 1910 confirmed the cultivation of chromogenic acid-fast bacilli from leprous lesions in symbiosis with amoebae at New Orleans. Duval (9) conceived that the rôle of the symbiotic amoebae and bacteria might be to break down proteins to simpler units that can be utilized by the lepra bacillus. He therefore added to various media either amino acids directly, or trypsin to produce cleavage of the protein molecule in the medium. On these media also Duval claims to have cultivated chromogenic acid-fast bacilli from leprosy.

EXPERIMENTAL DATA

In my first series of cultural experiments the technic of Clegg was essentially followed. The material from leprous lesions, which consisted of swabs from nasal or other superficial lesions containing many lepra bacilli, was inoculated into pure mixed cultures of amoebae and *Bacterium coli* growing on Musgrave and Clegg's medium. In a small percentage of these cultures acid-fast bacilli developed after several weeks at either incubator or room temperature. When these acid-fast bacilli were isolated in pure culture, they grew readily on ordinary nutrient media and produced pigment varying from yellow to orange in color.

In a second series of cultural experiments the material from leprous lesions was inoculated on the Musgrave and Clegg medium without the symbiotic amoebae and colon bacilli. A growth of chromogenic acid-fast bacilli developed in about the same proportion of these cultures as in the previous series containing the amoebae and colon bacilli.

The third series of experiments consisted in transplanting from the primary colonies of the pleomorphic partially acid-fast diphtheroid of Bordoni-Uffreduzzi, growing from leprous lesions on glycerine agar plates, into tubes of Musgrave and Clegg's medium. Again in about the same proportion of these cultures the chromogenic acid-fast bacillus developed in pure culture, the diphtheroids in the remaining cultures dying and disintegrating.

It will be recalled that in a previous paper (1) I showed that a pleomorphic partially acid-fast diphtheroid, similar to the Bordoni-Uffreduzzi diphtheroid from leprosy is the only bacillary organism that can be cultivated from smegmal secretions on glycerine agar. The fourth series of experiments consisted in transplanting from the primary colonies of this smegmal diphtheroid, growing on glycerine agar plates, to tubes of Musgrave and Clegg's medium. Chromogenic acid-fast bacilli, indistinguishable from Clegg's bacillus, developed in a small proportion of these cultures, the diphtheroids in the remainder of the cultures disintegrating.

In an endeavor to obtain further light on the source of Clegg's acid-fast bacillus, swab cultures were taken from the nasal cavities of a series of healthy persons and inoculated on Musgrave and Clegg's medium. A growth of acid-fast bacilli was obtained in a small percentage of these cultures. These acid-fast bacilli, however, showed certain differences from those cultivated from leprous and smegmal sources. While the acid-fast bacilli isolated from leprosy and smegma praeputii are chromogenic and grow in the first cultural generation as short chains of cocco-bacilli, those isolated from non-leprous nasal secretions produce no pigment and grow in the first cultural generation as single short rods.

Finally, the diphtheroids in the swab cultures from non-leprous nasal secretions were isolated by plating on glycerine agar. These diphtheroids in every case were the so-called Hoffmann's diphtheroid (*Corynebacterium pseudodiphthericum* Eberson). The primary colonies of this diphtheroid were transplanted from the glycerine agar plates to tubes of Musgrave and Clegg's medium. In a small proportion of these cultures, there developed a growth of acid-fast bacilli which, as in the case of the mixed cultures from the same source, consisted of single short rods and produced no pigment.

DISCUSSION

The results of my cultural experiments confirm the claims of Clegg and his successors that a chromogenic acid-fast bacillus can be cultivated repeatedly from leprous lesions on Musgrave and Clegg's medium. My experiments, however, demonstrate conclusively that neither the symbiotic amoebae of Clegg nor the protein split products of Duval are necessary for the development of these acid-fast cultures. The essential factor for the initial growth of these organisms appears to be the lean medium of Musgrave and Clegg which contains only traces of nutrient materials. The primary growth of this chromogenic acid-fast bacillus in my experience, never takes place on ordinary nutrient media; but when growth has once been established on the lean medium, it can be transplanted and will grow on ordinary nutrient media.

The growth of Clegg's acid-fast bacillus in cultures inoculated from single colonies of Bordoni-Uffreduzzi's diphtheroid onto Musgrave and Clegg's medium is susceptible to only one of two explanations. Either the chromogenic acid-fast bacillus has developed from the pleomorphic partially acid-fast diphtheroid, or Clegg's bacillus is a contamination. At the present stage of the investigation it would be rash to venture a positive opinion on this subject, and all that I shall attempt is to present the available evidence for each alternative.

The evidence supporting the theory that Clegg's bacillus is a contaminator is largely negative in character. We are not readily disposed, notwithstanding the considerable recent literature on life histories and mutations among bacteria, to accept the transmutation of one bacterial type to another. Second, the fact that Clegg's bacillus does not develop in all or even a majority the of cultures of leprous material and transplants of the cultural diphtheroid from leprosy, but only occasionally, might indicate that it is a contamination with a widely distributed saprophytic acid-fast organism capable of growing on Musgrave and Clegg's medium.

On the other hand, the evidence that Clegg's bacillus is a developmental stage of Bordoni-Uffreduzzi's diphtheroid, while more positive in character, is not conclusive. First, Clegg's acid-fast bacillus has been cultivated repeatedly in transplants from single colonies of Bordoni-Uffreduzzi's diphtheroid on Musgrave and Clegg's medium. Second, since Clegg's bacillus grows readily when transplanted to ordinary nutrient media, it should, if it be a contamination, appear as frequently in the plate cultures of leprous material and in the transplant cultures of Bordoni-Uffreduzzi's diphtheroid on glycerine agar as in cultures on Musgrave and Clegg's medium. On the contrary, while Clegg's bacillus had developed more or less regularly in 5 to 10 per cent of the cultures on Musgrave and Clegg's medium, it has never appeared in the many hundreds of plate and transplant cultures on glycerine agar and other nutrient media. Third, a chromogenic acid-fast bacillus similar to Clegg's bacillus develops in a certain proportion of the cultures of the pleomorphic diphtheroid from smegma praeputii when transplanted to Musgrave and Clegg's medium. And lastly, the consistently different types of acid-fast bacilli developing on Musgrave and Clegg's medium from leprous material and the cultural diphtheroid from leprosy, on the one hand, and, on the other hand, from non-leprous nasal secretions and the diphtheroid from such secretions, is apparently not consistent with accidental contaminations, but would appear to support the belief that in each case the particular type of acid-fast bacillus had developed from the respective diphtheroid.

SUMMARY AND CONCLUSIONS

The cultivation of a chromogenic acid-fast bacillus from leprous lesions on Musgrave and Clegg's medium is confirmed.

Neither the symbiotic amoebae of Clegg nor the protein split products of Duval are necessary for the growth of this organism.

The essential factor for the development of Clegg's bacillus appears to be the lean medium containing only traces of nutrient substances.

Clegg's acid-fast bacillus develops in transplants from colonies of Bordoni-Uffreduzzi's partially acid-fast diphtheroid on Musgrave and Clegg's medium.

A chromogenic acid-fast bacillus, similar to Clegg's bacillus, develops in transplants from colonies of the pleomorphic diphtheroid from smegma praeputii on Musgrave and Clegg's medium.

A non-chromogenic acid-fast bacillus, having also morphological differences from Clegg's bacillus, develops in cultures from non-leprous nasal secretions and from Hoffmann's diphtheroid isolated from such secretions, on Musgrave and Clegg's medium.

Clegg's bacillus appears to be a developmental stage of Bordoni-Uffreduzzi's diphtheroid, but the possibility that it may be a contamination can not as yet be absolutely excluded.

Both Clegg and Bordoni-Uffreduzzi and their successors may have cultivated different forms of one and the same pleomorphic bacterium from leprosy.

This organism appears to be identical with or closely related to the pleomorphic and facultative acid-fast so-called *Bacillus smegmatis.*

SUMARIO Y CONCLUSIONES

Se comprueba el cultivo en el medio de Musgrave y Clegg de un bacilo cromogénico y ácido-resistente de las lesiones de lepra. Para su desarrollo no se necesitan ni las amebas simbióticas de Clegg ni los productos de división protéica de Duval. El factor esencial para el desarrollo de bacilo de Clegg es el material magro que contiene pocas substancias nutritivas. El bacilo ácido-resistente de Clegg se obtiene de los trasplantes de colonias difteroides y parcialmente ácido-resistentes de Bordoni-Uffreduzzi ya desarrolladas en el medio de Musgrave y Clegg. Un bacilo cromogénico ácido-resistente, parecido al de Clegg, crece en el medio de Musgrave y Clegg cuando se hacen trasplantes de colonias del difteroide pleomórfico obtenido del esmegma del prepucio. En cultivos de las secreciones nasales no leprosas y del difteroide de Hoffman aislado de las mismas secreciones, se desarrolla en el medio de Musgrave y Clegg un bacilo ácido-resistente, sin cromogénesis, que presenta diferencias morfológicas del bacilo de Clegg. El bacilo de Clegg parece ser una forma evolucionaria del difteroide de Bordoni-Uffreduzzi aunque la posibilidad de que también pueda ser una contaminación no puede ser excluída aún. Ambos Clegg y Bordoni-Uffreduzzi, y sus sucesores, han cultivado distintasform as del mismo bacterium pleomórfico de la lepra. Este organismo probablemente es idéntico o está muy íntimamente relacionado con el *B. smegmatis.*

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THE ACUTE FORM OF AMERICAN TRYPANOSOMIASIS: NOTES ON ITS PATHOLOGY, WITH AUTOPSY REPORT AND OBSERVATIONS ON TRYPANOSOMIASIS CRUZI IN ANIMALS[1]

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INTRODUCTION

American trypanosomiasis (trypanosomiasis cruzi) is an endemic disease in some parts of Brazil, and is due to the action of *Trypanosoma cruzi*, which was discovered by Carlos Chagas in 1909.

The disease occurs in an acute form, which, when not fatal, becomes chronic, and the individual thus affected may live in a more or less invalided condition until old age. This is readily explicable when one has a knowledge of the pathology of the disease, which will be briefly summarized here; this summary will be followed by a detailed description of the pathology of the acute form. In other papers I will deal with the pathology of the chronic form in some of its aspects, and with the disease as manifest in animals spontaneously and artificially infected.

Transmitted by the bite of the *Triatoma megista* the trypanosome enters the circulating blood, from which it can be recovered during the first twenty to thirty days of the disease. The incubation period is probably brief, judging from the occurrence of cases in young infants, and from analogy with laboratory infections in animals. During this period, when the parasite is in the peripheral blood, the patient shows subcutaneous edema of a peculiar type, general glandular enlargement, enlargement of the spleen and liver, irregular fever, nervous phenomena, and sometimes bronchitis and cutaneous lesions. Chagas finds enlargement of the thyroid constantly in acute cases, and has reported cases with keratitis. If the patient dies during this period it is with the symptoms of cardiac insufficiency or those of meningo-encephalitis. In case the patient survives the acute stage the parasites disappear from the peripheral circulation at the end of the thirty-day period and are subsequently found only in the tissues. In chronic cases the parasite affects chiefly certain systems, thus giving the basis for Chagas' classification of the chronic cases into cardiac, nervous and pluriglandular forms.

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[1] This work was done at the Oswaldo Cruz Institute, Rio de Janeiro: Director, Dr. Carlos Chagas.

It is important to draw attention to the widespread distribution of the parasite and its lesions in the different viscera in the acute cases. These acute cases occur almost exclusively during the early years of life, in infancy or early childhood. Chagas has shown this fact to depend upon epidemiological factors related to the habits of the transmitting insect and to the method of life of the people in the endemic zone.

Through the courtesy of Dr. Carlos Chagas I had the opportunity of examining the tissues of a number of cases of this disease during the period of nearly four years that I spent at the Oswaldo Cruz Institute in Rio de Janeiro. At the same time I have studied the changes in experimental animals that will be referred to in this paper. I am here using the report of the autopsy that I performed on an acute case of the disease to serve as a basis for an exposition and discussion of the pathology of this acute phase of the disease.

The following report of an autopsy performed upon an acute case of American trypanosomiasis (Chagas' disease, trypanosomiasis cruzi) is published in order to show the character of the lesions that are to be expected in such a case. As the general process of the acute stages of this disease is similar in all cases, differing only in the intensity of the involvement of the different organs or systems, this protocol may serve to show the features of this process as exemplified in acute human cases. The microscopic examination of the most of the organs is appended. The case is a typical one and this is the first detailed report of a single acute human case to be published. Chagas has published brief protocols of autopsies in acute cases, and some phases of their histology have been dealt with by Vianna, Torres and Pinheiro Chagas. Other cases may vary slightly from what is here set down as the standard for the disease, but this variation is only one of intensity of infection and consequent greater or less severity of structural alterations.

In the study of Chagas' disease this case is important inasmuch as it shows the great similarity of the process to that which has already been reported by Vianna (1), Chagas (2), Torres (3), Rocha Lima (4), and others in experimental cases, and also as it explains the clinical phenomena that have been described in such great detail by Chagas.

AUTOPSY REPORT

The case is one of an eight months old, female mulatto, from Lassance, who died in the hospital of the Oswaldo Cruz Institute at 9:30 a.m. February 26, 1920, and was autopsied at 2:00 p.m. on the same day. She had been ill for about one month with fever, general swelling, and general glandular enlargement, chiefly of posterior cervical, with enlargement of the spleen and liver. During the last day she had interrupted respiration with forced expiration and diminished resonance over the bases of the lungs. On the night of the day before death she was agitated and became somnolent toward morning, dying at 9:30 a.m. Just before death she had a convulsion and died in it, and rigor mortis set in at once. (These clinical notes were kindly given me by Dr. Eurico Villela.)

Anatomic diagnosis. Chagas' disease (trypanosomiasis cruzi); peritonitis serosa subacuta; pericarditis serosa acuta; pleuritis subacuta serosa; myxoedema; lymphadenitis cervicalis (anterior et posterior), axillaris, mesaraica, lumbaris, iliaca, inguinalis et cruralis; hypertrophia et dilatatio ventriculi sinistri cordis; hypostasis pulmonum; petechiae pericardii, pleurae et capsulae hepatis; hyperplasia glandulae thyreoidicae; hyperaemia et degeneratio adiposa hepatis; hyperplasia et hyperaemia lienis; medulla ossea rubra.

The body is that of a female, dark-skinned, Brazilian infant, 66 cm. in length. Rigor mortis is slight in the smaller joints and the larger joints are flexible. There is slight hypostasis in the dependent parts.

The body has a well nourished appearance. On palpation of the surface of the body there is a peculiar sensation of firm elasticity of the skin and subcutaneous tissues, and definite pitting is not present. The swelling seems to be especially marked in the extremities and over the face but is not confined to these parts. The mons veneris is extremely prominent but there is nothing else notable in the external configuration of the body. The cervical, axillary and inguino-crural nodes are all palpably enlarged. Over both lateral portions of the occipital bone, between the mastoid area and the mid-line, there is an edematous swelling without discoloration of the skin. In the right posterior axillary region the skin shows several small (1 mm.), white points without surrounding change. The eyes, ears and nose appear normal. There is no icterus.

On section there is a considerable amount of orange yellow subcutaneous fat. This fat is moist but there does not seem to be free fluid in the interstices of the fat; rather there are bullae or blebs of swollen fat masses which give a peculiar oleaginous, almost gelatinous, appearance and feeling. This character of the subcutaneous fat is appreciable at all points at which it is examined. The muscles are moist and red.

The peritoneum is pale, glistening and moist. In its dependent parts is found a little more than 15 cc. of fluid. This fluid is clear, greenish yellow, without visible floccules at the time of removal, and seems to have a consistence and refraction more like that of whole plasma than of serum. There is no fibrin found in the peritoneum. The coils of intestine are somewhat distended and the serosa appears pale. The stomach is contracted and retracted beneath the liver, which extends between 4 and 5 cm. below the right costal margin. The dome of the diaphragm stands at the fourth intercostal space on each side.

Thorax. On removal of the sternum the precordial area appears large and the lungs do not meet over the distended pericardium. The thymus gland is small, soft and fleshy, being of a pink color. Fluid of the same character as that in the peritoneum is present in the pleural sacs. Six cubic centimeters of this were pipetted from the left pleural sac, and 3 cc. from the right. These quantities represent almost all that was present. There is likewise no fibrin nor floccules seen in this fluid at the time of removal.

Heart. The pericardium is distended, and on opening it this distension is seen to be due to the presence of fluid as well as to the enlargement of the heart. Thirteen cubic centimeters of fluid were pipetted from the pericardium, and this has the same macroscopic characters as the fluids already described. The inner surface of the pericardium is smooth and pale. The heart itself appears distinctly enlarged and the apex is rounded and formed for the most part by the left ventricle. There are a few pinpoint hemorrhages found in the epicardium over the right auricular appendix and in the right auriculoventricular groove. The vessels over the heart are not especially engorged. Mixed clot is present in both sides of the heart. The endocardium is smooth and transparent in its entire extent, showing no areas of dullness or opacity. The valves are all thin and pliable and no vegetations are present. The right side of the heart is not dilated and the wall of the right ventricle is not appreciably thickened. The columnae carneae are not enlarged. The left ventricle is large, distinctly more so than normal, and its cavity is spherical. The wall of the left ventricle appears slightly thicker than normal in spite of the dilatation. The heart muscle is firm, compact, cloudy and red. No hemorrhages or focal lesions are appreciable in the heart muscle. The base of the aorta shows no changes or anomalies, nor do the coronary arteries.

Lungs. The pleurae are smooth and glistening and there is no fibrin upon them. On the anterior surfaces, especially in the region of the interlobar grooves, there are small petechial hemorrhages, none exceeding 3 mm. in diameter. The lungs are crepitant in their entire extent and no nodules are palpable. The anterior parts of the lungs are pale and almost bloodless while the posterior parts are dark purple. On section the anterior and upper parts are pale, smooth and dry, and the posterior parts are smooth, moist, dark red, and drip blood. No exudate can be scraped from the surfaces. The vessels are free. The larger bronchi are empty and their mucosa is pale. The peribronchial nodes are not altered.

Organs of the neck. The tongue shows no change. The tip of the epiglottis shows some slightly injected vessels. The larynx is pale. The pharynx shows no recognizable change. The faucial tonsils are small and pale. The mucosa of the trachea is pale and there is no excess of mucus. The deep cervical lymph nodes along the trachea are distinctly enlarged, soft, moist, and red. The esophagus shows no change.

Thyroid gland. The thyroid gland is enlarged to almost twice its normal size, the enlargement being uniform in both lobes. The organ is firm and pale. The cut surfaces are of a light brown color and fleshy, and no material of a distinctly colloid character is recognizable. There are no nodules, cysts, or calcified areas in the organ.

Spleen. The spleen is enlarged and preserves its shape after removal. It measures 8 x 4.5 x 2 cm. There are no adhesions. The capsule is steel blue, smooth and transparent. The cut surface is dark red and of shagreen appearance. The pulp is not easily scraped from the cut surface. The lymphoid tissue is prominent in the form of numerous, closely placed, pearly nodules, but the individual nodules do not appear appreciably enlarged. The trabeculae are visible only with difficulty.

Adrenals. These appear unaltered in size and general appearance. The cortical layer is yellow and the medulla unrecognizable except at one small area in the right adrenal where it is pearly white.

Kidneys. These are unaltered in size. The capsule strips easily from a smooth, pink surface which shows remnants of fetal markings. The surface vessels are not prominent and are scarcely recognizable. The consistence is firm. On section the cortex and medulla bear normal topographic relations. The cortex is pale with a yellowish tinge and appears opaque. With a hand lens (× 5) the glomeruli appear pale. The peripheries of the pyramids are purple and their central parts paler. The mucosa of the calices, pelves and ureters is unchanged.

Urinary bladder. This is contracted and empty. Its mucosa is pale, with prominent rugae on account of its contracted state. There is no hemorrhage nor prominence of vessels.

Uterus and adnexae. The uterus appears normal in all respects as do the Fallopian tubes. The ovaries are of an elongated oval shape measuring 22 x 5 x 4 mm. They are firm and of a slate color with a reddish tinge.

Liver. This is somewhat enlarged uniformly, measuring 14 x 8 x 5 cm. The capsule is thin, transparent and glistening, showing a few punctate hemorrhages along its anterior lower border. The outer surface is smooth and pink mottled with paler yellowish areas. The consistence is firm but the liver substance is rather friable. The cut surfaces are pink mottled with yellow and abundant blood drips from them. The lobular markings are indistinct but in some parts small red points surrounded by narrow pale zones which in turn are surrounded by darker zones can be seen. There are no focal lesions seen. The gallbladder is small and contains a small amount of light green bile.

Pancreas. The pancreas is small and shows some redness of the interlobular tissue. The parenchyma is pale and the lobules small.

Stomach. The stomach is contracted and contains a sour milky fluid. The mucosa is pale.

Intestine. The serosa is pale and the wall of the small intestine is thin. There are no fecal contents. In the upper ileum there is an excess of mucus and the mucosa is reddened. The mucosa of the lower ileum and colon is pale and smooth. The lymphoid tissue is visible but not unduly prominent. There are no parasites found.

Lymph nodes. The cervical nodes have been described. The mesenteric nodes are large, soft, red, and moist, both externally and internally. They form a large mass almost completely filling the mesentery and individual nodes reach a size of 1.5 x 1 cm. The lumbar and iliac nodes are similarly enlarged. Scarpa's triangle on each side and the axillary spaces are filled up with masses of nodes of a similar character and size, but the nodes are all discrete from one another and show no alterations in their capsules nor adhesions, either with one another or with surrounding tissues.

Medulla ossea. A portion of the femur was removed and the medulla ossea appeared red and moist, but the bone was so small as to make examination of much of the medulla impracticable.

Brain. On removal of the scalp there are found small, red, moist nodes with some surrounding edema over the lateral plates of the occipital bone midway between the mastoid processes and the midline. The cranium is well developed and the fissures and fontanelles closed. The dura mater is not adherent, thin and pale. There is some congestion of the vessels in the pia-arachnoid and perhaps a slight increase of clear fluid in its meshes. It was impossible to collect this fluid without contamination with blood. The brain is well formed and the brain substance appears pale and moist. On section of the brain substance there apears to be a prominence of bleeding points. No focal lesions can be discerned. The ventricles are not dilated and the ependyma is smooth.

At the time of autopsy smears were made from all fluids and from all organs. These were fixed in absolute alcohol and later stained by the Giemsa stain. Careful search showed some trypanosomes in the smears from the heart but none were found in smears from any of the other organs.

The fluids found in the peritoneum, pleurae, and pericardium were removed carefully in sterilized pipettes without contamination with blood and were put at once into sterilized centrifuged tubes. These were kept in the icebox over night and were centrifuged the following morning. Very little sediment was obtained. Smears of the sediment were made from each tube and these were stained by Giemsa. Trypanosomes were found in each of these fluids, but not in great numbers; these trypanosomes corresponded in morphology to the *T. cruzi*. The cells constituting the sediment were lymphocytes and large mononuclear or endothelial cells in about equal proportion, as ascertained by differential count. Some red blood cells were present, but in relatively small numbers. No other cellular elements were found. The fluid was then examined as to its specific gravity and albumin content, with the following results (examination made by Dr. José Carneiro Felippe):[2]

	PERITONEAL	PERICARDIAL	LEFT PLEURAL	RIGHT PLEURAL
Specific gravity at 15°C.....	1,0227	1,0217	1,0206	1,0179
In 1000 cc. of fluid coagulable proteins..................	39.50	37.25	34.75	24.20

HISTOLOGIC EXAMINATION AND OBSERVATIONS

Microscopic examination of the heart. In contrast to the relatively slight macroscopic changes seen in the heart, microscopic examination shows intense changes in this organ. On first sight one is struck by the presence of the large number of foreign cells between the muscle fibers. The muscle fibers themselves have also suffered severe changes which will be first described. The cardiac fibers are for the most part rather widely separated from one another, giving a rather open appearance to the structure of the sections. Many, in fact the majority, of the fibers themselves show no special alteration in their structure. Others show a distinct increase in size with obliteration of the striations and an accentuated affinity for eosin, having an opaque, homogeneous appearance. This represents a hyaline or waxy degeneration. Other fibers, when stained with iron-hematoxylin, are seen to be distorted, and to have lost their striation and all internal architecture, while the sarcous elements are collected in masses and balls of various shapes in the different parts of the cell, taking the stain very intensely. In and about these cells I have been able to find no parasites, but the cells thus altered break into fragments, and large mononuclear leucocytes can be seen with large portions of these necrotic fibers in their protoplasm. Necrosis of the fibers and their phagocytosis in this manner is rather widely distributed. Fatty change in the fibers is not pronounced.

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[2] Dr. Felippe tells me that he examined the blood serum of this patient five days before death and found the proteins to be 58.30.

Parasitization of the cardiac muscle fibers is present but is not nearly as abundant as is the case in some other hearts that have been studied. The parasites are seen in the rounded form of leishmania as is usual with this parasite in the tissues (fig. 2). They are within the muscle fibers and are placed in rows along the central axis of the fibers or in rounded groups which may be so large as to cause a bulging of the sides of the fibers. Here they do not appear to disturb the cell, as no alteration in its structure is visible. The fibrils are displaced laterally and no evidence is given of structural alteration. It has not been my fortune to find free parasites in this heart, nor parasites within phagocytes as has been seen in hearts of other acute cases.

The interstitial tissue is the seat of the most intense change, and this is manifest by the presence of enormous numbers of foreign cells. These cells are present about the vessels and also far from the vessels, so that there is no impression given of a special perivascular arrangement of the cells (fig. 1).

Some of these cells are lymphocytes but the majority are of the type of mononuclear wandering cells. These latter cells are of various shapes, larger than the polynuclear leucocytes, with fairly abundant nongranular protoplasm with a slight affinity for eosin, and rather large nuclei sometimes centrally placed and sometimes eccentrically. The nucleus is either rounded or indented and presents a varying quantity and distribution of chromatin. In some the chromatin is abundant about the margins of the nucleus with masses projecting toward the center of the nucleus, while in others there is a delicate spongy network of the chromatin crossing the nucleus in all directions but leaving abundant achromatic substance. In this latter type the nucleolus is usually easily visible. I have grouped these cells under the heading of mononuclear wandering cells in spite of their various

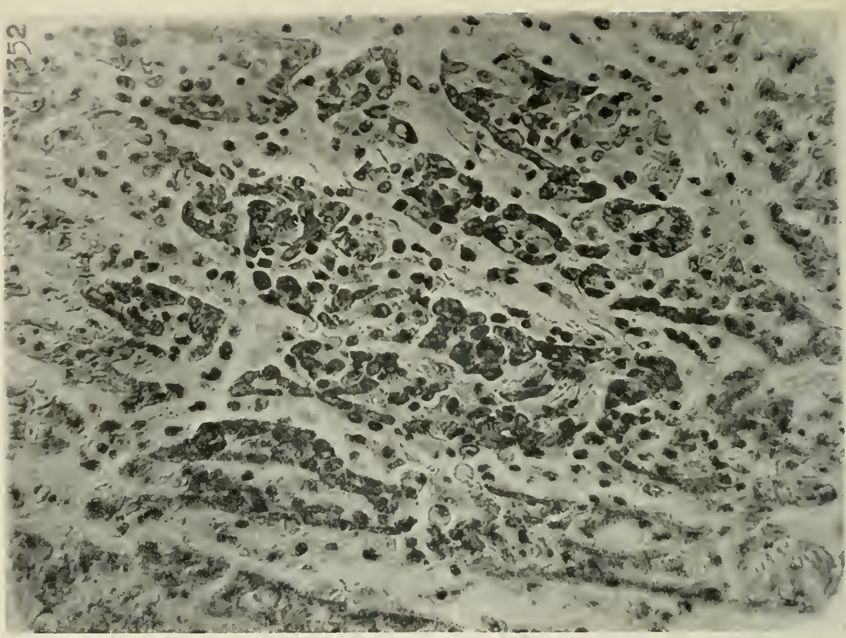

FIG. 1. T. CRUZI. HEART SHOWING GENERAL REACTION IN ACUTE HUMAN CASE

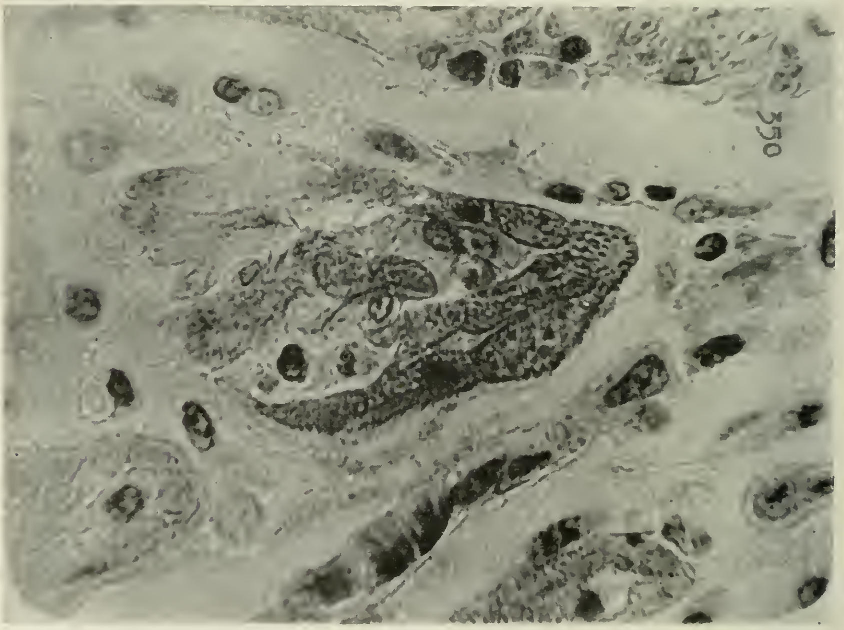

FIG. 2. T. CRUZI. HEART. PARASITIZED CARDIAC FIBER

appearances, and will so refer to them. Their origin may be from endothelial cells or possibly from reticular connective tissue cells, but there is evidence in these sections of their multiplication in the tissues. Their phagocytic properties are pronounced. Lymphocytes and erythrocytes and nuclear fragments are frequently seen within their protoplasm and they may be seen surrounding and engulfing portions of necrotic muscle fibers.

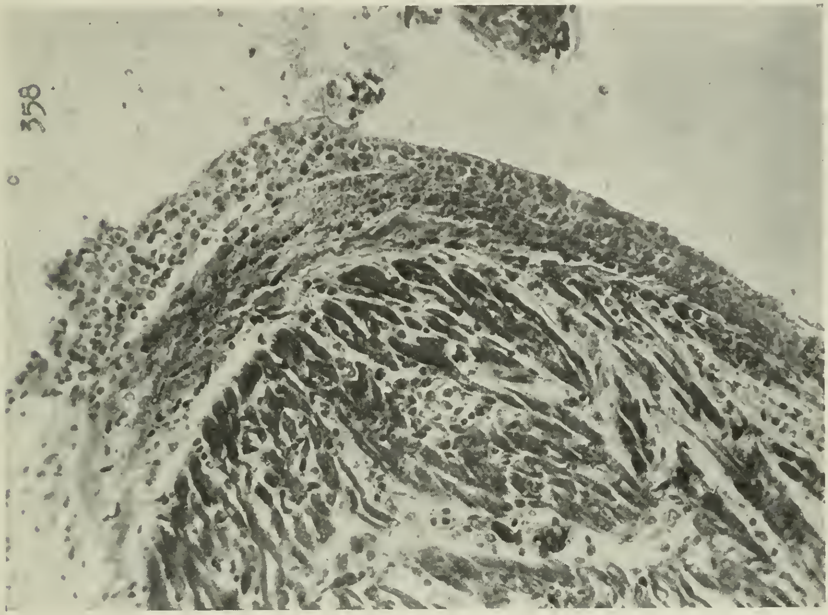

Fig. 3. T. cruzi Human Infection. Infiltration of Endocardium

The endocardium in some places shows marked infiltration of the same nature, so that at the places affected there appears a fairly dense mass of these mononuclear cells forming a band six or eight cells deep. For the most part the endothelial cells lining the endocardium appear unaltered. These areas of endocardial infiltration are small, especially covering the columnae carneae, but nevertheless must represent areas of inflammatory reaction. Parasites were not encountered in these foci in the sections (fig 3).

Similar foci of infiltration are present in the epicardium and in the subepicardial fat. While parasites have not been found in these epicardial foci, their presence is to be postulated in as much as they were encountered in the pericardial fluid.

These findings corroborate and extend the work of Chagas, Vianna and M. Torres on the human heart. In the study of the minute changes in the heart of the acute form Torres described the direct mechanical effect produced in the fibers by the parasites, as well as hyaline, granular and fatty degeneration of the fibers.

In diseases due to other trypanosomes cardiac lesions such as above have not been described. In an Ayrshire bull which was infected with *T. dimorphon* Wolbach and Binger (5) have described the trypanosomes in the connective tissue of the intermuscular septa, between the muscle fibers, in the lymphatics, and within phagocytic cells, and infiltration of the endocardium and myocardium with endothelial, lymphoid and plasma cells. Clark (6) has found piroplasma in smears from the heart in animals affected with piroplasmosis.

The lesions above described in the heart of this case form the most prominent feature of acute cases of Chagas' disease, being present in all cases with varying grades of intensity. The lesions are readily reproduced in animals, especially young dogs, which form excellent material for the study of the acute stage of this condition.

In cases that successfully pass from the acute into the chronic stage, characteristic lesions of a less destructive character are found in the heart, and the parasite may also then be found. In these cases the primitive cardiac bundles are affected in the same way as the myocardium, and, indeed, the parasite seems to have a predilection for these regions, and the changes here produced give rise to the train of severe clinical manifestations that have been described in detail by Chagas and Villela (7). The pathology of this chronic cardiac lesion will furnish material for another article, it sufficing here to state that lesions diagnostic of the disease occur in these hearts.

As regards the pathogenesis of the cardiac lesions probably more than one factor is concerned. The parasites multiply within the muscle fiber until they distend the sarcolemma to such an extent that its rupture occurs. The ruptured fiber is thus converted into a foreign body to be removed, of which we have evidence in its phagocytosis of mononuclear cells. Torres postulates a disturbance of the circulation to account for some of the changes in the muscle fibers that have been described. Toxins play an important part in the pathology of diseases due to other trypanosomes, and there is evidence of a toxin from this trypanosome in the fever, ecchymoses in serous membranes as described here, severe degenerative lesions in the liver, and proliferative changes in spleen and lymph nodes in which parasites are not demonstrable. Rocha Lima has considered that the toxins play a part in the production of the cardiac lesions in animals, and Pinheiro Chagas has shown that the hepatic lesions in acute human cases are best explained on the basis of the action of a toxin. It seems reasonable to suppose that both mechanical and toxic elements account for the cardiac lesions in this case.

Lungs. Sections of the lungs show an interesting condition. The sections of the bronchi of medium and smaller size are without change. In some of the bronchioles there are seen a few mononuclear cells. In examining a section of the lungs with low magnification one sees small areas which are more compact than the well-aerated surrounding parts, and one gains the impression of a bronchopneumonia, but examination with a higher magnification shows this not to be the case. In some of the alveoli there are very large foamy cells with many fat-like vacuoles and large, pale eccentric nuclei of the same type as occur in the lung outside of caseous tuberculous foci. But the chief change is in the interalveolar and interlobular septa, which are very much thickened on account of the presence of many mononuclear cells with a few polymorphonuclears. This is not accompanied by any fibrosis; but gives rise to a certain amount of atelectasis of the alveoli concerned. Parasites were searched for unsuccessfully.

Spleen. The splenic pulp is much engorged with well preserved erythrocytes and the venous sinuses are dilated. Here and there throughout the pulp are seen isolated large cells with abundant eosinophilic protoplasm and large, nearly circular nuclei rich in chromatin. The Malpighian follicles are large and in large part are made up of proliferating cells of the germinative centers, interspersed between which are numerous endothelial cells. Only at the margins of the follicles do the lymphocytes form a distinct zone. The proliferated germinative cells assume various shapes and sizes and have a very irregular arrangement with relatively loose structure. In some of the follicles are seen small rounded bodies, about half the size of an erythrocyte, with an inconstant quantity of chromatin which is usually distributed in a very narrow zone at the extreme periphery of the body,but not completely encircling its circumference. These same bodies are more numerous in the lymphoid follicles of the lymph nodes, and their origin and nature has not yet been decided upon.

Some of the smaller vessels show the same filling of their walls with large mononuclear cells, as has been seen in the similar vessels of the brain.

Parasites were searched for through two sections with the oil-immersion lens, but were not found. Other sections were also searched unsuccessfully with lower magnifications.

Studies on the histology of the spleen in this disease have not been of a detailed nature, and are rendered somewhat difficult on account of the frequent presence of malaria. Chagas states that clinically and anatomically enlargement and engorgement of the spleen are constant features in acute human cases. Vianna made no record of the histology of the spleen in his article. Rocha Lima notes that he found few parasites in rare cases among his experimental animals. It is a rarity to find parasites in the spleen in human cases. On the contrary, puppies infected with some strains of this trypanosome show an exceedingly large number of parasites in the enlarged spleen. The parasites may abound in all parts of the spleen and lie in the endothelial cells of the sinuses. Whether they are present in other cells or not I am unable to say, for the parasitized cells are most frequently so filled and distorted by the parasites as to obscure their exact nature. Megakaryoctes, such as described by Pettit in his experimental infections with other trypanosomes, frequently occur in the spleens of artificially infected animals, but I have not seen them as yet in human spleens.

Lymph nodes. The lymph nodes from the various parts of the body were examined, and all show essentially the same appearance. The general architecture of the node is preserved, with its peripheral follicles and central medullary part well recognizable. The vessels are dilated and filled with erythrocytes; this congestion is notable in all of the glands examined. The cortical lymphoid nodules are rather large, with a definite zone of well-formed lymphocytes at their periphery, while the centers are made up of a much looser structure in which the cells are of various types. In these central zones there are some lymphocytes and some larger cells which correspond to those described as germinating cells or lymphoblasts. In addition there are many larger cells of the large mononuclear type such as will be described as so abundant in the sinuses, and there are many circular masses of chromatin which represent nuclear rests. The large mononuclear cells frequently contain yellow pigment (hemosiderin), erythrocytes, lymphocytes and nuclear fragments that have been phagocytized. This general appearance of the cortical nodules is such that an impression of central necrosis of the nodules is gained and there is also a definite hyperplastic process as well.

The lymph sinuses that surround the cortical nodules and those that are between the medullary cords of lymphocytes are filled with some lymphocytes and many large mononuclear cells that are obviously of endothelial origin. These are of the same type as those seen in the centers of the cortical nodules and show a very active phagocytosis. They are seen to contain red cells, hemosiderin, lymphocytes and nuclear fragments. Some of these cells have two or even more nuclei.

The important processes seen in these glands appear to be the congestion, lymphoid hyperplasia, mononuclear cell production and phagocytosis, with red cell destruction and a destruction of other cell elements that are phagocytized. Careful search through many sections has failed to reveal trypanosomes.

Cells that could be interpreted as megakaryoctyes or megaloblasts have not been found.

This condition of the lymph nodes is the rule in the acute human cases. That it is sufficiently characteristic to justify an etiologic diagnosis on histologic evidence alone I am not prepared to say. It differs but little from the condition of the nodes described in African sleeping sickness where the terms "cells of Marschalkô" and "morula cells" have been introduced to indicate peculiar cells derived from lymphocytes.

The lymph nodes in African sleeping sickness contain the parasites in such numbers as to make them valuable from the diagnostic standpoint, whereas such is not the case in Chagas' disease. It is a great rarity to find parasites in sections of human lymph nodes at any stage of the sickness. But in animals experimentally infected with some strains of *Trypanosoma cruzi*, the lymph nodes, like the spleen, may show exceedingly large numbers of the parasites infesting cells that correspond to those that contain the parasites in the spleen. Megakaryocytes are frequent in the lymph nodes of some experimentally infected animals, and they often contain trypanosomes.

Chagas has emphasized the constancy of the enlargement of the lymph nodes in acute human cases, and the fact that all the nodes of the body are affected.

Liver. The chief, and practically the only, change seen in the liver consists of a fatty change, which is present throughout the organ but is most marked in the peripheries of the lobules about the portal spaces. Surrounding the portal spaces there is a zone in which the hepatic cells are almost replaced by fat, each cell presenting one large globule of fat surrounded by a very narrow rim of cytoplasm with the nucleus pressed to the side of the cell. The cells of the central and middle portions of the lobules also show fatty change but in a lesser degree, the fat here being present in the form of numerous small globules. Aside from this fatty change the liver cells show no alteration. There is no undue hyperemia of the liver, nor is there any recognizable alteration in the interstitium. There is no such collection of lymphocytes or mononuclears about the portal spaces as has been described in other human or experimental trypanosomiases. Parasites were not found.

Chagas has drawn attention to the constancy of the enlargement of the liver in acute cases of the disease and the extreme fatty change that it undergoes. Pinheiro Chagas (8) has made the liver the subject of a special study, basing his work on observations made on thirteen animals, four acute human and five chronic human cases. This work is of such merit that I will here quote his conclusions. He says:

> In experimental cases of Chagas' disease the regressive changes in the liver are manifested by a more or less intense and more or less generalized parenchymatous degeneration. At times the degenerative process goes to the extreme of necrosis, which may be of only a few liver cells or of more extensive portions of the lobules in their central or medial zones.
>
> These changes depend on a trypanotoxin liberated on the death and disintegration of the parasites. In acute human cases the action of this trypanotoxin is revealed in the liver by a marked fatty change of an infiltrative character. The various forms of these regressive changes are accounted for by the greater or less activity of the toxin liberated. The focal necroses with polymorphonuclear infiltration observed in some experimental cases may have a mechanical explanation in the state of hyperplasia of the Kupffer cells or in the formation of red thrombi. The iron pigment seen with such frequency in these cells results from the destruction of red cells. The presence of parasites in the liver is not a common occurrence. When present, they by preference parasitize the Kupffer cells where they form more or less developed agglomerations which are represented at times by the leishmania-like bodies and at times are formed of adult flagellates.
>
> The *Trypanosoma cruzi* may be placed beside the trypanosomes studied by Pettit as among the most active in producing hyperplasias of the hemolymphatic apparatus.
>
> In the liver of animals inoculated with *Trypanosoma cruzi* and in human cases of American trypanosomiasis this hemolymphatic activity is manifested by the appearance of a considerable number of lymphoid cells that are generally organized in small foci or in partial sheaths for the branches of the portal vein. In chronic human cases observed, the necrotic process of the liver is associated with the myocarditis produced by the *T. cruzi*.

I am able to corroborate the observations and the conclusions of Pinheiro Chagas in all respects. I have had one series of puppies inoculated with a strain of trypanosomes that produced a more abundant parasitization than he worked with, and have more liver cells proper showing the parasites; but even in these cases there are more Kupffer cells than true hepatic cells attacked.

Kidneys. Sections of the kidneys show very little change. There is slight tumefaction of the cells of the convoluted tubules, and a small amount of granular matter in some of them. There is no alteration in the glomeruli nor in the interstitial tissue. Parasites were searched for unsuccessfully. Fat is not present, as shown by Sudan III.

Renal lesions appear to be uncommon in this disease. Vianna simply noted that he had seen inflammatory reactions in the kidney. Chagas does not emphasize renal lesions. I have seen one acute case that presented an albuminuria.

In my puppies I have found large numbers of the parasites in the unstriped muscle and in the endothelial lining of the veins of the capsule of the kidney, and in the branches of these venules that pass into the organ. Deeper in the organ they have also been found in the lining of the veins and in the endothelium of the capillaries of the glomerular tuft. Fairly well marked degenerative changes are present in the epithelial cells of the convoluted tubules and some of the glomerular tuft cells are vacuolated. The kidneys merit further study of a more intensive character.

Urinary bladder. Parasitized muscle fibers are found in the muscular walls of the bladder. In one of these fibers there are 30 units recognizable in one plane. About the parasitized fibers there is no reaction. In some of the muscle bundles where parasites are not recognized there is slight infiltration with mononuclear cells between the individual fibers, but this is so slight as to be practically insignificant in amount. More marked is the presence of numerous isolated mononuclear cells in the areolar tissue and also between the epithelial cells of the mucosa.

No other reports of vesical involvement have been found.

Pancreas. The pancreas shows no change and no parasites. Lesions of the pancreas in this disease have not been recognized.

Thymus. No notable change is found in this organ. It is moderately vascular and shows no regressive changes nor evidence of inflammation. The reticulum cells are relatively prominent. Many sections were examined in search of parasites, but without success.

The thymus has not been studied by previous workers nor have I made a detailed study of it in many cases. In a number of my artificially infected puppies the parasite is present in large numbers in endothelial cells and free in the tissues, and these glands also show changes similar in general character to those noted in spleen and nodes.

Tonsils. The chief changes found in the tonsils are in the lymphoid follicles which show a considerable activity in the presence of large, pale-staining cells and nuclear remains. In the extrafollicular parts of the tonsil similar large cells are found isolated one from the other, and the whole picture is remarkably like that encountered and already described in some of the lymph nodes. No parasites have been found in this organ.

At the present time I am unable to give further information concerning the tonsils, except to say that they have not been especially studied by previous workers, and that some of my puppies showed an intense parasitization in this organ.

Thyroid gland. Sections of this gland show the regular formation of acini separated into groups by rather abundant fibrous tissue. This fibrous tissue is distributed in a regular manner throughout the gland, dividing it into lobules of approximately equal size, and there is no cellular infiltration of the gland. There is no undue engorgement of the vessels and there are no foci of infiltration with lymphocytes. The acini are small, circular or oval, and their epithelium is low cuboidal. There are only a few of the acini in the sections that show the formation of colloid material, and, where present, this is pale. Many of the acini show many desquamated epithelial cells in their lumen. In some sections near the periphery of the gland there are solid masses of epithelial cells, these masses being isolated from one another and of about the size of the formed acini. Some of the acini show slight infolding of the epithelium into the lumina of the acini, and a piling up of the epithelium into more or less solid masses, but the cells are always of cuboidal shape. There is a complete absence of the formation of high cylindric epithelium such as is seen in cases of active hyperplasia, and there is almost complete absence of the formation of colloid. The latter is characteristic of the thyroid during infantile life, and such evidences of growth as have been here described are those which are to be expected in a growing infant of this age.

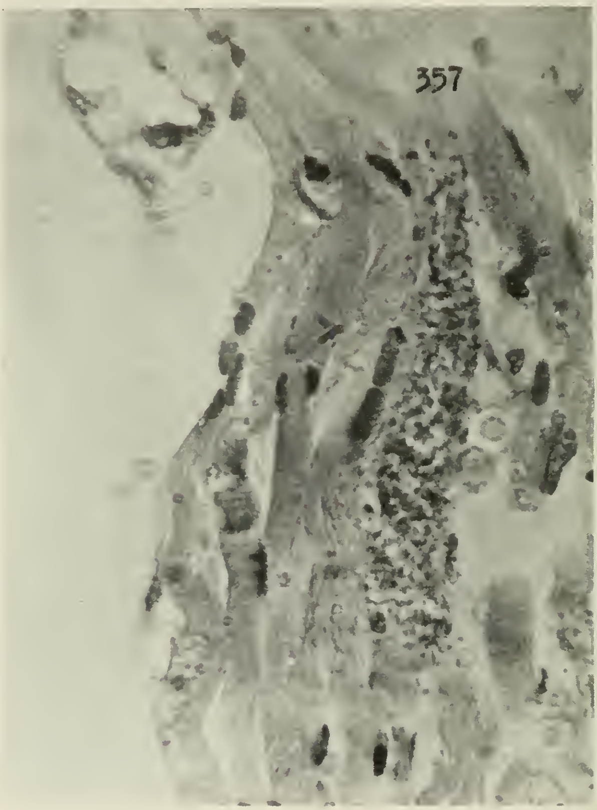

Fig. 4. T. cruzi. Leg Muscle. Muscle Fiber Parasitized

Chagas first described this disease as a parasitic thyroiditis, but soon found that the trypanosome produced other, and probably more important, changes in the body. However, it is by no means disproved that in the endemic zone of the disease the involvement of the thyroid may lead to serious disorders, with cretinism as a result. My own studies of this gland have not been sufficiently extensive to allow me to make any statement based on personal observations. In the present acute case of the disease the thyroid was enlarged. In animals I have found numerous parasitic collections in the endothelial cells of the capillaries and venules of the thyroid, giving sufficient enlargement of these cells to occlude the vessel. In one of the acute human cases studied at the Instituto Oswaldo Cruz parasites were

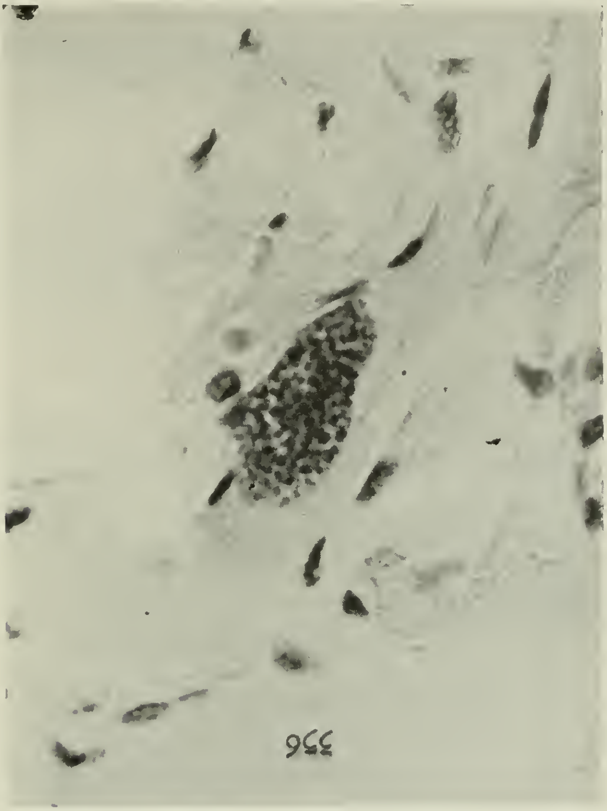

Fig. 5. T. cruzi. Subcutaneous Tissue. Muscle Fiber Parasitized

found within the cells lining the vesicles. In chronic cases extensive sclerosis, cysts, struma, and adenomata have been found. The pathogenesis of these alterations in the thyroid in the chronic cases merits the closest study.

Ovary. Sections of the ovaries show no inflammatory infiltrations nor exudation. The cortex is very closely packed with primitive follicles, only a few of which have gone on to the stage where they are surrounded by high folicular epithelium. The great majority are still in the stage where the epithelium about them is very low cuboidal, almost flat, and many of the follicles still contain two or three ova. In some of these primitive follicles with more than one ovum there can be seen the commencement of further subdivision of the follicle. The impression is gained that there has been a delay in the formation of the Graafian follicles. The stroma of the ovary shows no change. Here and there can be seen a deeply staining mononuclear cell, but these are always isolated and represent the normal wandering cells seen in almost all tissues.

Parasites were found in this organ. One mass of the rounded form was encountered in a fiber in the wall of an arteriole. Another is seen in a cell at the periphery of one of the primitive follicles. The mass in the wall of the arteriole is in a muscle fiber in the middle coat. There are about fifteen of the parasites in the rounded tissue form in this mass, the nucleus of the cell is pushed to one side, and the outline of the cell is destroyed (figs. 8 and 9).

Chagas has reported the finding of the parasite in the ovary in one other human case and in animals. From his clinical work in the endemic zone Chagas concludes that there is evidence of hyperfunction of the ovaries, but seems not to think it proved that this is a result of direct action of the trypanosome on the ovary.

In the testicle and epididymis of guinea pigs Vianna has described extensive changes due to the presence of the *T. cruzi*, and also found a trypanosome in the cephalic end of a spermatozoon. He further found parasites in the spermatic fluid of guinea pigs which is sometimes ejected at the time the animals are being killed.

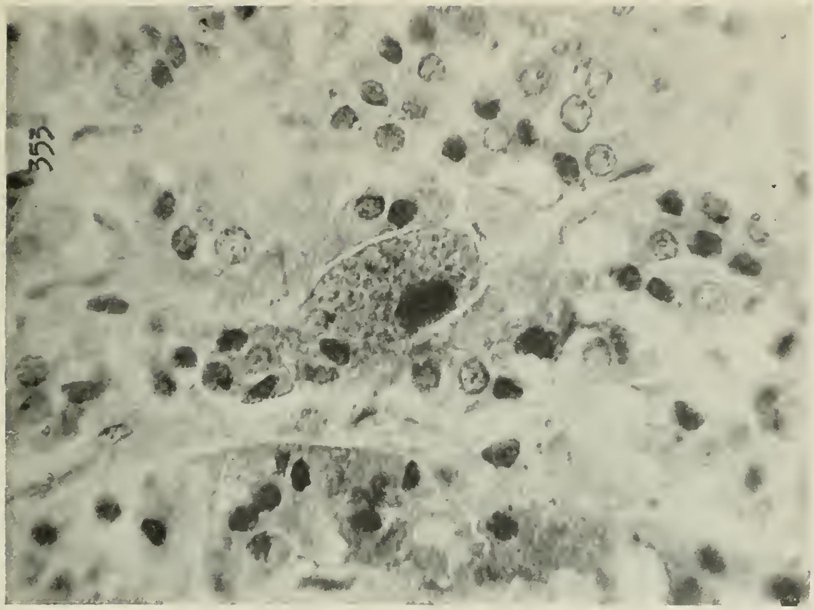

Fig. 6. T. cruzi. Adrenal with Cortical Cell Parasitized

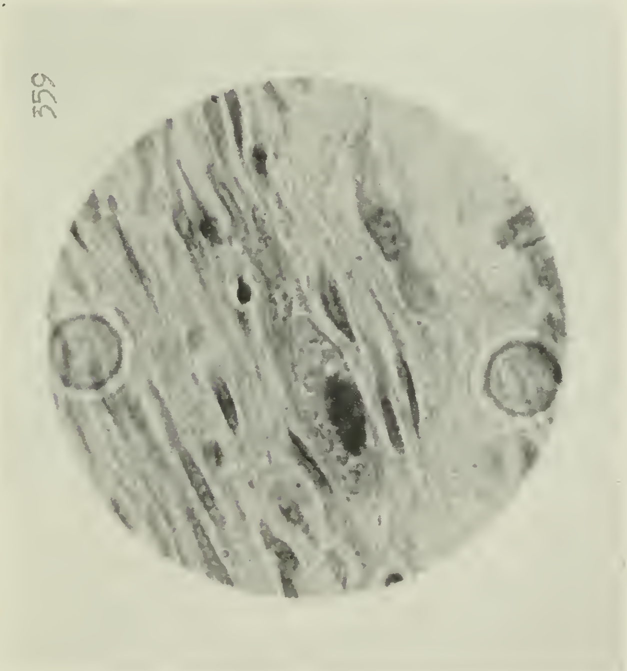

Fig. 7. T. cruzi. Uterus with Infection of Muscle Fiber

Uterus. In sections of the uterus a muscle fiber is found parasitized with numerous examples of the rounded form. The muscle fiber itself and the nucleus are well preserved and there is no reaction in the vicinity. In the rest of the myometrium and endometrium no alteration is found (fig 7).

Rocha Lima has found the parasite in the placenta in an animal. Nathan-Larrier, and later Eurico Villela, showed hereditary transmission of the infection in dogs. In two previous autopsies on acute human cases the parasite has been found in the muscle fibers of the uterus.

Suprarenals. Parasitized cells are found in the zona fasciculata of the cortex. These cells are very much enlarged and contain a large number of rounded forms of the parasite. Isolated parasites are not found. A few foci of mononuclear infiltration are seen in the cortex, but in these it has not been possible to find parasites. No other change in the suprarenal has been recognized (fig. 6).

Chagas has emphasized the involvement of the suprarenals as well as of other endocrine glands in this disease. Vianna described "lesions of the cortical cells and inflammatory foci" in a chronic human case, and found parasitization of a cortical cell in a guinea pig similar to the one described in this case. In my own animals parasitization of the suprarenals has not been infrequent, but recognizable histologic lesions have not been marked. Chagas mentions the finding of the parasites in the cortical cells of the suprarenal and states that the medullary zone has shown considerable reduction in volume. He also says that clinically there are groups of cases showing signs of suprarenal insufficiency with pigmentation of mucous membranes and skin, some bronzing of the skin, general asthenia, exaggerated somnolence and rapid emaciation.

Muscle. Sections were examined from the skeletal muscles of the leg, abdomen and neck. A few parasitized fibers were found, and in these the parasites are arranged in a bead-like chain in the middle of the fiber and along its long axis. These parasitized fibers show no anatomic change. In examining a section of muscle the attention is attracted by small foci of cellular infiltration among fibers. These are composed of mononuclear cells which are not in the immediate proximity of vessels and in none of these have I succeeded in finding parasites. At these points the muscle fibers are separated from one another by the accumulation of cells (figs. 4 and 5).

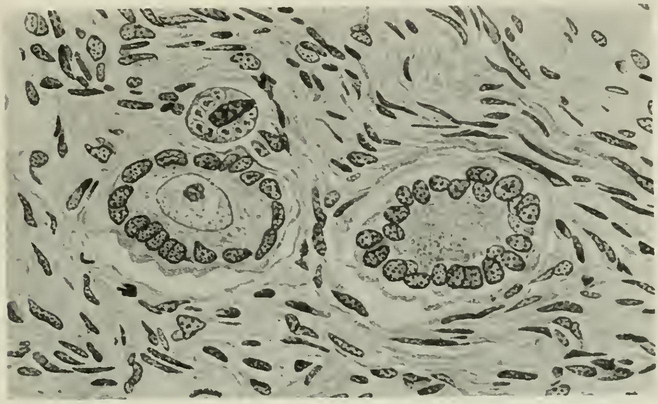

FIG. 8. T. CRUZI. OVARY WITH PARASITES NEAR GRAAFIAN FOLLICLE

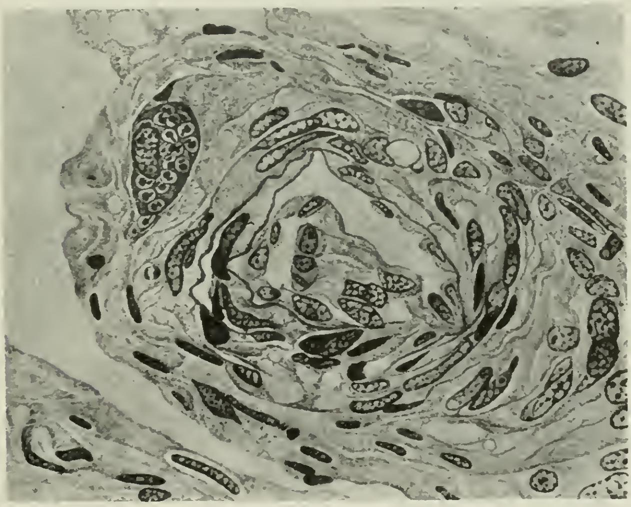

FIG. 9. T. CRUZI. OVARY WITH PARASITES IN WALL OF SMALL VESSEL

Much detailed study of the effect of these parasites on muscle has been reported by Chagas, Vianna, M. Torres and Rocha Lima. In man and in animals muscle is one of the favorite sites of localization of the *T. cruzi*, which multiplies within the fiber and eventually ruptures it, setting free the rounded and the flagellated forms. The muscle fibers are extremely tolerant of the presence of the parasites within them, showing practically no change other than displacement of their fibrils until rupture takes place, and it may well be, as Torres maintains, that this mechanical rupture of the fibers forms the prime cause of the inflammatory reaction that may reach extreme degrees in severe infections. Polymorphonuclear cells and mononuclear cells, as in the case here described, may phagocytize fragments of muscle and liberate parasites.

Brain, cord and meninges. The leptomeninges over the hemispheres and at the base show a slight thickening and a slight infiltration with mononuclear cells and lymphocytes; while this process is not severe in any part, it is fairly uniform in distribution and unmistakable.

Throughout the brain and cord there is generalized congestion. The principal change that at once attracts the attention is the presence of masses or collections of cells that can be seen with low magnification. As far as can be determined by short series of sections these foci bear no direct relation to the blood vessels. They can be traced through several consecutive sections. These collections consist of cells of the type of neuroglia cells mixed with some mononuclear cells from which they can be distinguished. These foci occur in the gray matter as well as in the white matter, and in the bulb and cord as well as in the hemispheres. Parasitized cells, which appear to be neuroglia cells, were found including large numbers of parasites. Only a few of these cells were found and they are near but not in the collections of cells above described. Within the collections of cells I have not succeeded in findings parasites. While only a few parasitized cells were found in the sections examined, there is no doubt that it would be only a question of patient searching to show that they are widespread throughout the brain. The fibroglia fibrillae do not appear to be increased, judging from the examination of appropriately stained sections, but this conclusion is subject to error on account of the time lapsing between the death and autopsy (fig. 10).

Convulsions within a few hours of death, such as occurred in this child, are very frequent in the acute cases. The parasites multiply within the central nervous system and produce widespread foci of encephalitis and myelitis such as are here described.

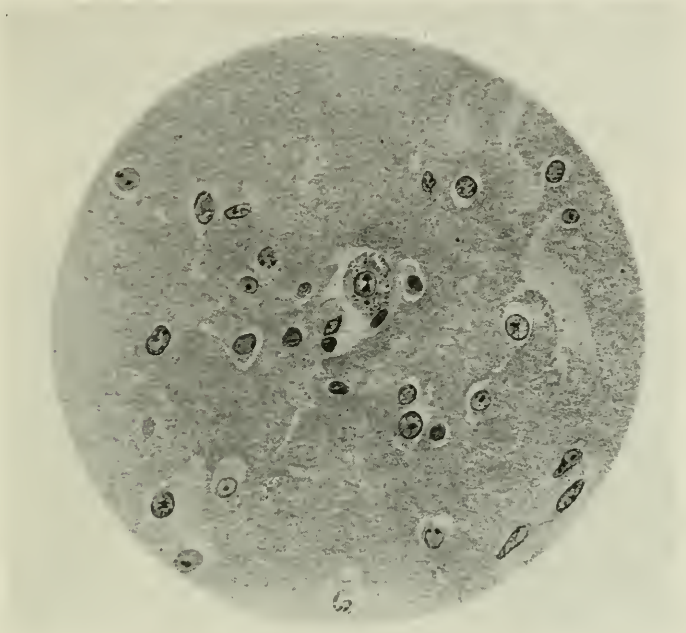

Fig. 10. T. cruzi. Brain with Parasitized Cell

If the disease does not prove fatal in the acute stage many of the survivors present symptoms that are accounted for by the localization of the parasite and the reactions to it in the nervous system. Inasmuch as the foci of encephalitis are multiple and may occur in any part of the central nervous system, various motor or sensory phenomena are to be expected, and these have been found and described by Chagas, who has also described various degrees of arrest of mental development and mental degeneracy.

Vianna was the first to describe the foci of encephalitis and myelitis in the human cases and to show that they are readily produced in animals. M. Torres and Villaca have studied these in more detail. The cells making up the foci of encephalitis and myelitis are neuroglia cells, and it appears that it is these neuroglia cells that become parasitized. The determination of the exact nature of the parasitized cells is rendered difficult by the distortion of the cell brought about by the presence of the trypanosomes within them. Parasitization of the nerve cells proper is practically never seen, but in the semilunar ganglion of a heavily infected puppy I found a parasitized cell that was unmistakably a gangion cell.

PATHOGENESIS

This trypanosome has been previously shown to possess morphologic and biologic characteristics which differentiate it from other trypanosomes. In its pathogenicity it also differs in some respects from the other trypanosomes, and this difference in large part depends upon the fact that it becomes a histoparasite and upon the types of cells that it infests. In trypanosomal diseases in general the changes produced are conceived as being due to the direct action of the parasites and to the toxins derived from them. In dealing with the action of the *T. cruzi* there also enters into consideration the actual destruction of fixed tissue cells (muscle fibers, etc.) caused by the multiplication of parasites within them to such an extent as to rupture the cells, and the sequent reaction to this destruction of tissue on the part of the wandering cells of the body.

As regards the types of cells infested by the *T. cruzi* we are dealing with derivatives of the mesoderm (muscle, fat endothelium, suprarenal gland, ovary, uterus, testis, serous membranes), entoderm (thyroid, thymus, liver, etc.), and ectoderm (ganglion cells and glia (?) cells). In severe cases of acute infestations all of these tissues may be parasitized and it is then difficult to consider the parasite as having a special predilection for any one tissue. However, in less severe infestations and in cases that become chronic the parasite undoubtedly shows a definite predilection for certain tissues and among these the muscle tissue, especially cardiac, nerve tissue and endothelium are prominent.

The trypanosome multiplies within these tissues even at a very early stage of the disease and soon (not usually later than the first month in human cases) disappears from the peripheral circulation. In spite of the fact that the organisms must reach the circulating blood again when freed by rupture of their host cells, they nevertheless disappear; this disappearance can best be explained by some constituent of the blood that is inimical to the life of the trypanosome.

DISCUSIÓN

Previamente se ha demostrado que este tripanosoma posee caracteristicas morfológicas y biológicas que lo diferencian de otros tripanosomas. En su patogenicidad también difiere en ciertos respectos de otros tripanosomas y esta diferencia depende en parte del hecho de convertirse en un histoparásito sobre las clases de células que infesta. Los cambios producidos por las enfermedades tripanosómicas en general se conciben que sean debidos a la acción directa de los parásitos y de la toxinas derivadas de ellos. Cuando se trata de la acción del *T. cruzi* debe considerarse la destrucción actual de las células del tejido fijo (fibras musculares, etc.) causada por la multiplicación de los parásitos dentro de ellas causando su ruptura y la reacción subsiguiente por parte de las células errantes del cuerpo.

Tocante a las clases de células infestadas por el *T. cruzi* se trata de derivados del mesodermo (músculo, grasas, endotelio, glandula suprarenal, ovario, útero, testis, membranas serosas), del entodermo (tiroide, timo, hígado, etc.), y del ectodermo (células gangliónicas y celulas glia). En casos severos de infestación aguda todos estos tejidos pueden ser invadidos por parásitos lo que hace difícil el creer que dicho parásito tenga predilección especial por algún tejido. Sin embargo, en infestaciones de poca severidad

y en casos cronicos, el parásito parece tener en realidad una predilección definida hacia ciertos tejidos como el muscular, especialmente el cardiaco, asi como tembién el tejido nervioso y el endotelio.

El tripanosoma se multiplica dentro de estos tejidos al principio de la enfermedad y pronto (durante el primer mes en los casos humanos) desaparece de la circulación periférica. A pesar del hecho de que los organismos se introducen en la sangre circulatoria al ser liberados mediante la ruptura de las células huéspedes, es de notarse que desaparecen de aquella. Esta desaparición se explica como debida a la presencia de alguna substancia en la sangre que es hostíl a la vida de los tripanosomas.

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BOOK REVIEWS

The Practical Hygiene of Native Compounds in Tropical Africa. Being notes from the experience of the first eighteen years of European work in the Katanga. By A. Pearson, M.B., B.S. Lond., Late chief Medical Officer of the Tanganyika Concessions and Union Miniere Du Haut-Katanga, and R. Mouchet, M.D., Late Assistant at the University of Liege; Late of the State Laboratory at Leopoldville; Chief Medical Officer of the Union Miniere Du Haut-Katanga. Publishers, Bailliere, Tindall & Cox, London. Price 22s. 6d.

This book is composed of twelve chapters dealing with General Considerations of Native Life, General Situation of Compounds, Construction of Huts in Compounds, Sewage Disposal and Latrines, Water Supplies, Food, Personal Hygiene, Hospital Organization, Recruiting, Epidemic Diseases and Pathological Notes.

This book fills a long felt want and is the first of its kind that has been printed of which we have knowledge.

The medical man intending to engage in work in tropical countries can well use this book as a valuable guide in his daily occupation.

The first chapter deals with the life of the native, giving one a good idea of his habits, telling us many things we should avoid in handling these individuals. It emphasizes the dangers of overcrowding.

The second chapter deals with the site one should select and the manner in which the buildings as huts, kitchens, latrines, etc., should be arranged. The sketches in this chapter are very valuable and give such detail that makes the newcomers work easy.

The third chapter deals with contruction of huts and tells us the best type one should build not only economically but for the health of the individual. Here also the cuts are of great value to those using the book.

The fourth chapter deals with sewage disposal and latrines. A water carriage system is evidently prohibitive, but it is no doubt the best. The fuming pit is looked upon as the best for natives. There is no doubt that this will keep out flies but the continual smoke and consequent irritation has its drawbacks and we believe that the fly-proof deep pit latrine is a better type.

The fifth chapter deals with water supplies. The dry chlorine method of water purification is used, but we believe the chlorine gas method would be better if transportation and availability would permit of its use.

The sixth chapter deals with food in a very interesting and valuable way, giving one a basis on which to work as to supply of food for natives under any condition.

The seventh chapter deals with cleanliness or personal hygiene in an interesting way, however, one wonders what milk oil fluid is. The use of arsenic to kill flies is always dangerous to life. We have other methods which are just as efficacious. The method of sticky wires to catch flies is a very excellent one. The chapter is well written and gives one a very good idea of the troubles of a sanitarian and how he may combat them.

The eighth chapter deals with hospital organization and gives a medical man an excellent idea as to the methods of handling sick natives both in the interest of employer and employee.

Chapter nine deals with methods of recruiting and emphasizes the detention barracks as an economic measure of great value. This chapter is of exceptional interest.

Chapter ten deals with epidemic diseases peculiar to the African native, the experience of the authors is very valuable to the new medical officer.

The results of vaccination as regards typhoid fever, shows to all of us the great value of this means of controlling this disease, especially in countries where this disease is being first introduced. The practically absolute control of this disease by vaccination among people of unsanitary habits is of inestimable economic value and should be given the widest circulation possible so that all peoples may be able to avail themselves of this method of disease prevention. Hereafter, in developing new countries, medical officers should insist on vaccination of the workers at the beginning of any enterprise.

From the methods now being tried out we believe that in the near future the same control of pneumonia by vaccine will be seen that now takes place with typhoid fever and smallpox.

The means used to rid the compounds of ticks again shows that it is much better to get rid of the source of disease than to try to cure it.

The last chapter gives a brief idea of the pathology found in these diseases.

The book is of great value and no medical man going into tropical work should be without it.

The authors are to be congratulated that they have seen fit to give their years of experience publicity so that all may derive aid from it.

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U. S. A. Medical Field Service School,
Carlisle, Penn.

Laboratory Studies in Tropical Medicine. By C. W. Daniels, M.B. Camb., F.R.C.P. Lond., and H. B. Newham, C.M.G., M. D. Durh., M.R.C.P. Lond., D.P.H. Camb., D.T.M. and H. Camb. Fifth edition. William Wood and Co., New York. Price $7.00.

There are 576 pages in the fifth edition of this well known book. This includes the index. In addition there are seven full-pages colored plates which are excellent. The 549 pages of text exclusive of one page of appendix which has to do with (*a*) the Antiformin Method for Tubercle and (*b*) Arrow Poisons, is divided into twenty-nine chapters. There are 184 illustrations in addition to the colored plates. These are on the whole well chosen and admirably complement the text. This fifth edition is increased by 16 pages over the fourth edition which came out in 1918 and by 192 pages over the first edition which came out in 1903.

In the preface to the first edition of "Laboratory Studies" the statement is made that the book is intended for the isolated practitioner in the tropics. Presumably the fifth edition is addressed to the same class of men rather than to the physician with a well equipped laboratory at hand. It is the reviewer's opinion that with some textual expansions this little book could have been made to meet the requirements of both the pure laboratory worker in the tropics and the isolated worker who must extemporize a laboratory. Indeed the authors seem to have grown away from the original idea as they have inserted articles on the care of "Experimental Animals" and on complicated procedures in serology, bacteriology and parasitology which comtemplate laboratory ability and laboratory discretion of a high order. In view of the fact that numerous tropical cities at the present time are equipped with laboratories capable of turning out work of a high order and that it is utterly impossible for a physician practicing in the tropics to get very far without knowing and using laboratory diagnosis it is our opinion that this useful book could have been made much more useful had the authors talked not only to the laboratorian who is learning to fly but also to him who has found his wings.

The first chapter deals with the laboratory and its equipment, the cleansing of slides and cover glasses, instruments and reagents and the care of experimental animals. The methods and material are simple, well chosen and adequately described. It would have been helpful had one of the machines for obtaining a blue Bunsen flame from gasoline been included, though the Automatic Bunsen burner for methylated spirit described and pictured on page 3 looks as if it would be indispensable to the isolated worker.

Chapter two deals with postmortems, the preparation and sectioning of tissues, the preparation of Museum specimens and the packing of specimens for shipment. As most cities in the Tropics have CO_2 cylinders available at all times it would seem a valuable addition to this chapter had the methods and apparatus for frozen section work been described.

Chapters three, four, five and six which deal with Normal Blood and Its Study, Abnormal Blood Conditions and the Blood Plasma do not seem to have been much altered in their material from the fourth edition. Some of the newer haemoglobinometers and counting chambers which are considered quite helpful at the present time are not included. Plate I facing page 102 shows Blood-Spectra compared with spectrum of Argand Lamp. It is excellent and the reader is greatly aided by having the legend immediately under the plate. With Plates V, VI, and VII, excellent though they are, it is necessary to turn back several pages in order to read the legends. Chapter 6 also has the description of the Wassermann reaction. The technique of Dr. F. E. Taylor is given. For tropical use it is wise in a work of this character to describe a technique and the theory of the original Wassermann reaction. Perhaps it is well to make this the quantitative test as the authors have done. As Noguchi's test will tell qualitatively as much about syphilis as any test ever devised and as it obviates the necessity for keeping the larger laboratory animals, it is particularly valuable for tropical use and it would seem to be a mistake to omit its description. In the reviewer's opinion Noguchi's test is the simplest one of all those which give a dependable answer to the question "does the man have syphilis."

Chapter 7 deals with Protozoa occurring in the cells of the blood. The material in this chapter is admirably handled and constitutes a most excellent guide to the study of the organisms of malaria and Leishmaniasis. It is no fault of the book but constitutes a regretable fact to say the least, that we do not get some other terms than "benign" for the organisms of simple tertian and quartan malaria; for while these usually do not kill people, they nevertheless have *no* qualities which, by any stretch of the imagination could entitle them to be considered as benignant.

Chapter 9 deals with trypanosomes and spirochaetes and does it admirably. The authors are apparently unwilling to accept a classification which puts spirochaetes under the bacteria; otherwise they would have considered them in the section devoted to bacteria. Though the organisms of syphilis, relapsing fever, yellow fever and allied forms are, from their morphology not typically protozoa, neither are they typically bacteria. The microgamete of malaria has not *all* the demonstrable addenda of a perfect protozoan but no one would seriously content that its protoplasm is bacterial in nature. From the fact that these organisms are flexible which bacteria are not, and from the fact that immunologically they are more like Protozoa it would seem logical to treat them as protozoa. Furthermore they act like protozoa in that many of them are killed in the body by chemotherapeutic agents (which is not the case with any known Bacterium) and some of them are transmissible only by arthropod intermediation and require a developmental period *in* the intermediary.

The recognition of the genus *Spironema* for the organisms of relapsing fever and the placing of the organisms of syphilis and yaws in this same genus, is in the opinion of the reviewer, a mistake. The genus *Borrelia* apparently has precedence over all other names for the relapsing group and the organism of syphilis is certainly as distinct in morphology as is *Leptospira* which the authors recognize. It would seem difficult to better the classification which places all these organisms under the *Proflagellata* and recognizes the genera *Borrelia, Treponema* and *Leptospira.*

Chapter 10 on Pigment Deposits and Degenerations in Tissues, Chapter 11 on Intestinal Protozoa and Chapter 12 on Certain Protozoa occurring in the Tissues leave little to be desired.

The chapter on the Urine, chapter thirteen, is good as far as it goes but it is not full enough. It would have helped this chapter if a discussion of albuminuria, glycosuria and a good kidney-function test had been described and the chapter supplemented by a description of laboratory methods used in the chemistry of the blood. There is a crying need for a better understanding of what happens to the chemical constituents of the blood in several tropical diseases. One questions the advisability, in a book of this character of giving the right of way to a description of the Diazo-reaction over a discussion of such essentials as those just mentioned.

Chapter fourteen on Bacteriology has been cut down slightly (1 page) over the fourth edition. There has been considerable rearrangement of this chapter. The chemical examination of water has been omitted as has also several cuts and some text on moulds and yeasts. The material on pure bacteriology and bacteriolic technique seems to have undergone little change from the fourth edition and one is a little disappointed to find no reference made to hydrogen ion concentration in the determination of the reaction of culture media.

The chapters from the fifteenth to the twenty-ninth inclusive take under consideration the following subjects; Measurement of Microscopic Objects, Helminthic Parasites—Cestodes, Helminthic Parasites—Trematodes, Helminthic Parasites—Nematodes, Faeces, Anthropoda, Insecta, Diptera, Mosquitoes, Classification of Mosquitoes, Dissection of Mosquitoes, Demonstration of Development of Parasites in Mosquitoes, Eggs, Larvae and Pupae of Mosquitoes, Fleas, Lice and Bed-bugs, Arachnida-Ticks, Mites, Porocephalus, Crustacea-Cyclops and Malaria Survey. The material in each of these chapters is brought up to date and it would be difficult to find any fault with the authors' method of treating these several subjects.

The authors' clear, succinct style in presenting their subject commends the book to the laboratory worker as does also the sequence of subjects. The printing, paper and binding are excellent and the book can be recommended as a dependable laboratory guide to the study of Tropical Diseases.

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AN INVESTIGATION TO DETERMINE WHETHER CLONORCHIASIS MAY BE DISSEMINATED ON THE PACIFIC SLOPE[1]

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Clonorchiasis is believed to be a chronic affection of the liver, in which that organ may be enlarged or atrophied, and in which in later stages of the disease, ascites is developed, the individual becomes emaciated, and the affection may progress to cause death. However, there are numerous instances in which evidences of the presence of the malady have been found in individuals enjoying apparent good health. Likewise, the liver has been found affected at necropsy examinations in which the immediate cause of death had no connection with this disease, and there was no clinical history which would suggest its presence in the individual. The literature contains descriptive case records and reports of experimental investigations, but our knowledge of the etiology and pathogenesis of the condition is incomplete in several respects.

The fluke, *Clonorchis sinensis*, has been observed living within chronically inflamed and dilated biliary passages of the liver of man and other mammals. Its presence in these passages seems to be constantly attended with chronic inflammatory changes in them. It has also been found in the intestine, but apparently unaccompanied with inflammatory changes in this organ. The ova have been recovered from the bile in the passages, or in the gall bladder, or in the intestinal contents. It is, therefore, probable that while living in these biliary passages, the fluke evacuates its eggs into the bile stream, and thence to the intestinal tract and fecal discharges of the patient. Investigations and experimental research of recent years suggest that the ova live in the fecal deposits for a sufficiently long period to permit of their further development into miracidial forms. Harujiro Kobayashi reported in 1911 that he had succeeded in developing the mature adult parasite in mammals by feeding the animals with portions of raw fish in which small encysted larvae were present. Later experiments indicate that the parasites become established in the livers of these animals, and are accompanied with changes in their bile ducts. These observations, when considered in the light of analogies presented by the life cycle of other trematodes will permit of the conclusion that this worm develops larval stages in at least one intermediate host and reaches its mature form in mammals.

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[1] Read, by invitation, at the nineteenth annual meeting of the American Society of Tropical Medicine, San Francisco, Calif., June 25, to 26, 1923.

This parasitism, then, is probably dangerous to the health of the individual affected, and is probably contagious or communicable. Further, the therapeutic efforts thus far published have not been effective in its cure. It may, therefore, be dangerous to the public health.

In accordance with the immigration act passed by Congress in 1917, the Surgeon General of the United States Public Health Service has classified the disease as a ''dangerous contagious disease.'' Such a classification makes all immigrants infested with *Clonorchis* mandatorily excludable from admission to the United States. This classification is believed to be justified in properly safeguarding the health of the public, pending more complete evidence of the efficacy of recent therapeutic efforts at cure of the individual, and of the mode of dissemination of the parasite.

The fluke has been reported prevalent in man and in the lower animals in southern and northern provinces of China, in Korea, and in Japan. Faust, after recent surveys, reports it prevalent among dogs about Peking, and in the Yangtze River Valley at Wuchang. However, he did not find a single case in man amongst 359 general dispensary patients examined at Wuchang, and but 1 case amongst 57 hospital patients carefully surveyed by the examination of an average of 3 stool specimens. Similar observations have been made by other observers in districts of China and Japan. The records of the San Francisco Immigration Station indicate that the natives of Quan Tung are at present heavily infested. Most of the Chinese immigrants arriving at San Francisco are from Canton.

In so far as has been determined, no investigator has reported the presence of clonorchiasis in a native of the United States, who has not lived some time in the Orient. But few cases have been reported in Europeans or natives of the United States. H. B. Ward is cited by Looss as having reported the disease present in the United States. M. J. White of the United States Public Health Service reported the presence of the fluke in the livers of 18 Chinese who died of plague in San Francisco during the years 1901 and 1902. Herbert Gunn reported, in 1916, its presence in Orientals arriving in this country, and in sick Chinese residents. W. W. Cort, in 1919, found it present in oriental inhibitants of California, during a survey made under the supervision of the California State Board of Health. In this survey of orientals working in the delta lands of the San Joaquin River, he found that 2 per cent of 500 individuals examined harbored *Clonorchis*. No reports seem to have been made of the presence of *Clonorchis sinensis* in other mammals in this country. However, H. B. Ward, in 1895, reported the presence of *Opisthorchis felineus* in cats in the United States. The species he found (*O. pseudofelineus*) was later determined to be distinct from the European form.

During the past two years 5775 Japanese have been examined at San Francisco, preliminary to admission to the United States and among these, 5 were certified as infested with *Clonorchis*. During this same period, 3701 Chinese were similarly examined and 315 found infested. Chinese ship crews examined have shown as high as 30 per cent infected. Some of these discrepancies are, in all probability, due to the examinations carried on previous to embarkation, since the immigration laws provide penalities for the common carriers which bring to our ports aliens who are mandatorily excludable. Earlier observers have found as high as 40 per cent infestations in natives about Canton. High degrees of infestation have also been found in some provinces of Japan, especially Okayama, from which some of the Japanese immigrants come. It will be evident that a marked contrast exists between the percentage of Chinese infested, amongst those seeking entrance to the country, as opposed to that reported from the Canton district, and to that found amongst Chinese ship crews. If the latter index may be applied to the former group, it will be seen that the sanitary restrictions of immigration procedures are effectively holding in check a probably copious seeding of our country with *Clonorchis* ova.

The infection is, of course, determined to be present in these individuals by stool examinations, and laboratory procedures such as those involved in the search of specimens of feces for the ova of helminths cannot be 100 per cent effective, even though carried out to a degree which is not practical. Nor can the administrative restrictions on individuals affected with such helminths be absolute. Individuals who harbor *Clonorchis* are, therefore, in all probability, being admitted to the country. In former years, they have undoubtedly been admitted, as was shown by the reports previously mentioned. Once admitted, they may be a source of infection and dissemination for an unknown time, probably for years. The longevity of the parasite in man has not been determined, though clinical histories and reports indicate probable infestation for eight years, and records at the Immigration Station at San Francisco suggest that this may be as long as twenty-five years. Masatomo Muto has found experimentally that the duration of life of the parasite in dogs is more than three years. During all this time, these dogs were evacuating ova of the parasite.

It will, therefore, be manifest that it is desirable and pertinent to learn whether the disease can be transmitted in the United States, since it may be necessary to establish procedures to prevent its spread within the country; or, on the other hand, if it cannot be spread within this country, the specific immigration restrictions are unnecessary, and may unduly damage the general welfare of the public by interfering with the normal social and commercial intercourse between the natives of the countries concerned.

The analogies offered in the development and spread of other trematodes suggest that the topography, climate and demography of much of the Pacific Slope would seem to offer favorable conditions for the spread of this fluke. In topography, climate and water courses, together with their fauna, many similarities with the Yangtze River Valley are presented. In this latter valley, *Clonorchis* is very prevalent. The Pacific Slope is traversed by two mountain ranges which form wide valleys watered by rivers subject to great variation in their flow, inundating large inhabited sections at one season, and contracting to relatively small courses during many months of the year. The climate on much of the slope is sufficiently warmed by the Japan current to be warm temperate or subtropical. Large numbers of oriental peoples, some affected with the disease, have settled in the agricultural sections, and are cultivating crops, such as garden vegetables and rice, which are common to those countries in which the fluke is endemic. Irrigation is practiced in many sections, and the methods in some of siphoning water from the rivers over levees and returning the runoff to the river would seem favorable to the development and spread of trematodes. Fish abound in these rivers used for irrigation, and are consumed in quantities by natives both of European and oriental origin, by swine and by dogs. The habits and customs of these oriental people are the results of practices which have been established by many generations, and are but slowly changed to those of the native American population.

Muto has reported that the larval form of *Clonorchis* occurs in Japan in the gastropod, *Bythinia striatula*, var., *japonica*. Kobayashi states that *Melania livertina* predominates in the waters of the area in Japan in which the disease is endemic. Both of these are operculated snails. In so far as is known, they do not inhabit the waters of the Pacific Slope in the United States. There are, however, representatives of the same groups such as *Amnicolinae*, *Lithoglyphinae* and *Goniobasis*. Several representatives of the *Limnophila* are also prevalent such as *Lymnei*, *Planorbis*, *Physa*, *Ancylus*, and *Pompholyx*. Faust states that the operculated snails abound in the waters of the

Yangtze River, and our survey has shown them prevalent in many of the fresh water streams of the Pacific Slope. One species of *Bythinia* is found in this country east of the Mississippi River.

The specific fish mentioned by Kobayashi as intermediate hosts have not been found in the waters of this slope, but several are prevalent which are allied to them, and among them *Cyprinus carpio*. The carp is wide spread throughout the fresh waters of the United States. It is used as food by some, and is fed to swine. The gold fish, found to be an intermediate host, has escaped into the waters of this country, and reverted to its dark color. It is probably sold as a food fish, under a different name.

In view of the above conditions, the investigation conducted by us thus far has been developed under two main divisions. The one consisting of a survey of mammals to determine the degree of dissemination of the fluke, the other involving the collection of the prevalent fresh water molluscs and fish, and experiments designed to accomplish the infestation of these animals with the larval forms of the parasite.

The presence of the fluke was sought by search for the ova in specimens of stool from man, and by examination of the liver and gall bladder with its content from the lower animals.

The examination of stools was made by agitating with a stick a portion about 2 cm. in diameter in approximately 60 cc. of water, and straining through a gauze of medium fineness into a centrifuge tube of 15 cc. capacity. This was centrifuged one minute at a moderate speed, the supernatant fluid poured off, and the sediment thoroughly washed twice by shaking in fresh tap water after each centrifuging. The final sediment is suspended in entirety on a slide, and the slide completely examined with a 16 mm. objective, using a mechanical stage. It has been found that the eggs settle very quickly after one washing. This method is the one in use at the Immigration Station at San Francisco, and has been evolved as one necessary to detect many of the carriers of helminth ova. Four hundred and seven individuals were examined, but no *Clonorchis* ova were found in them. Of these, 391 were of European stock and 16 orientals, among them 12 Japanese native born children living in the delta country of the San Joaquin River, California. About 2 per cent of those examined harbored other worms, including 1 case of *Dibothriocephalus latus* infection. These people were patients of public general hospitals, and many of them of races which consume much fish.

It would seem that the disease would become established in the cats, dogs or swine of the country before being established in the people. The findings both of Askanazy and Rindfleisch in East Prussia on *Opisthorchis felineus* and of Faust at Wuchang and at Peking, in his examination of mammals, lend color to this conclusion. At Wuchang he found about 30 per cent, and at Peking about 70 per cent of the dogs examined harbored this fluke. Whereas he found but one human host. Since in addition to the dog and cat, the hog has been found harboring this parasite, it may serve as an index of its prevalence. In certain sections of the Pacific Slope, the hogs are frequently fed on fish and fish offal. However, the examination of 1033 livers and bile, and 435 samples of bile from the gall bladders of hogs, in which the liver was not examined, revealed no infestation. These livers were selected because of some macroscopic pathology, and represent but a small percentage of a very large number of hogs subjected to inspection by trained veterinary officers of the United States Department of Agriculture. Likewise, the examination of 38 dog livers and 8 cat livers, and 10 dog feces was negative. The swine, dogs and cats examined were from the delta country of the San Joaquin River, California. It was in this district that Cort found 2 per cent of the orientals examined were infested with *Clonorchis*. The livers were examined by section through and along the bile passages and gall bladder. The entire content of the gall bladder was collected, diluted 1 :5 with tap water, sedimented in tall cylinders for forty-eight hours, and the sediment centrifuged and examined as outlined above. It was found that ova would settle to the bottom of cylinders prepared as indicated within twelve hours, and the eggs were readily recovered by centrifugalization of the sediment in the cylinders.

It is the belief of several authors that the miracidia of this and similar parasites hatch spontaneously and are free swimming until they are ingested by a host, or until they invade the host. Experimental studies have been made to determine whether the ova will hatch spontaneously. Conditions of temperature, acidity, pressure due to depth of water, and chemical content of the water have been varied without positive results. These results conform with those of other students of the subject. Spontaneous hatching seems very rarely to have been noted. Numerous efforts have been made to observe motile free swimming miracidia, but these have likewise been negative.

During the study of the ova and miracidia, in conjunction with C. W. Stiles, it was noted that the egg capsule, or shell, is embossed with a mosaic-like pattern. This feature was a constant finding in specimens of several weeks age, as well as in freshly collected specimens and does not appear to be an artefact. This mosaic arrangement gives the surface of the egg a somewhat mammillated appearance on section.

Attempts have been made through several months to infest the snails native to the waters under investigation. Snails have been kept in aquaria heavily seeded with specimens of feces containing tremendous numbers of ova, which were apparently alive. No cercaria has been found thus far in snails hatched during the experiments. Nor have cercaria, which have a probable relation to the development of *Clonorchis*, been found in snails collected and kept in the laboratory, under temperature and feeding conditions which would seem to be favorable to the development of these larva. However, some monostome and distome cercariae, and rediae have been observed.

Our experiments show that the ova are ingested by the snails, and in some species, most of the ingested eggs dehisce, with the escape of the miracidium. Ova maintained in a menstruum containing the juice made by grinding some of these snails in tap water did not dehisce, but eggs subjected to rubbing in a small rubber tube containing sand were dehisced, many with the escape of the miracidium. Snails of the species in which dehiscence of the eggs usually occurs have present in the strong muscular ventriculus-like organ several grains of sharp-edged sand. The food passes through this organ in its route along the alimentary canal, before reaching the liver. The grinding of the ova in these "gizzards" may have opened them, or some chemotactic juices may have induced the miracidium to emerge. As previously stated, however, none of the snails with which we have experimented during the past nine months has been demonstrated to contain cercariae which were probably larval forms of *Clonorchis*.

Young carp have been kept both in basins into which ran the overflow from aquaria heavily seeded with ova and snails, and in the aquaria, but without any positive findings of sporocysts within them.

The temperature conditions maintained throughout the experiments have been those of the water delivered through the city mains of San Francisco, and the atmospheric conditions out-of-doors, the water varying from 7° to 19°C. and the atmosphere from 5° to 25°C. During the past six months, additional aquaria with water at temperatures varying between 14° and 29°C., with room temperatures from 18° to 28°C. have also been under observation. The factor of temperature in the development of the larval forms of *Clonorchis* may be of importance. After his survey of the animal parasites of verbrates of north China, and considering the climatic conditions of that section, Faust was led to the conclusion that "Practically speaking, trematodes do not occur in man from infections acquired in north China." Cort found experimentally that temperature variations had a definite influence on the escape of one species of cercaria from its snail host.

Most of the rivers of the Pacific Slope are fed by the snow covered Sierras, and have relatively rapid and short courses, so that their waters are cold throughout much of the year.

DISCUSSION

It would appear that with temperatures prevailing in the water probably unfavorable to the development of the parasite; and with the absence of those snails and fish which predominate in the waters of those oriental districts in which the disease is endemic, that the dissemination of the fluke would be improbable. The probability would seem further reduced by the administration of the sanitary regulations of the Pacific states which prevent the fertilization with raw human feces of large tracts devoted to rice culture and garden vegetable culture. Further reduction may be accomplished by the infrequency of the consumption of raw fish among natives of this country of European stock.

However, it is observed that numerous cercariae do develop in the snails of the pacific slope rivers and sloughs. The snails themselves prevail in their natural habitats in waters ranging from 20° to 2°C.

It is common knowledge that adaptation to changed environment frequently occurs amongst the lower animals, and splendid analogies seem to be offered in the habits of Schistosome larvae, and in those of *Opisthorchis felineus*, as noted by various observers. The Schistosome larvae have been found developing in several different members of *Limnophila*. *Opisthorchis felineus* will develop in two or more fish of the same family but different genus, and 12 different species of fishes are reported to harbor *Clonorchis sinensis*.

Though generalized fertilization with raw human excrement is not practiced on a large scale, river water is irrigated over lowlands intensively cultivated and on which are many habitations, and the seepage and excess water returned to the rivers. These conditions are conducive to enteric affections, since in some of these sections, typhoid fever is a seasonal disease. Also much inhabited and cultivated land is inundated each year during the rainy season, and the flood waters return to the main streams abounding in fish. Rice culture is aided by fertilization with treated sewage, and the runoff from these fields is returned to the rivers.

Though the practice of consuming raw fish is uncommon in this country, much poorly cooked fish is undoubtedly eaten. *Dibothriocephalus latus* infestation is found not infrequently in various sections of the country, including this coast. Askanazy and Rindfleisch in their investigations in East Prussia established the fact that fish tape-worm infestation was frequently accompanied by *Opisthorchis felineus* infestation, a condition simulating closely in its pathology that of *Clonorchis sinensis* infestation. Ward has reported the presence of *O. pseudofelineus* in cats of the United States, which would indicate that the conditions are present for the dissemination of this latter fluke. With the presence of a species of *Bythinia* and the wild goldfish in the waters of the eastern section of the country, it would seem that some of the biological conditions believed necessary to the life cycle of *Clonorchis sinensis* are also present in the country.

SUMMARY

The results of this incomplete investigation may be summarized as follows:

Clonorchiasis, a chronic affection, probably communicable, is classified under the immigration laws as a dangerous, contagious disease, and all aliens afflicted with the disease are mandatorily excludable.

The disease is present, and has been present in oriental natives of this country for a period of at least twenty years. It is prevalent in that section of China furnishing most of the present Chinese emigrants to the United States, and it is present in an appreciable percentage of those Chinese who apply for admission to the United States.

No reports have been made of the infestation of man or the lower animals who have resided continuously in the United States, nor has infestation been found during this survey in California of 407 people, 38 dogs and 8 cats, and 1468 hogs from a district in which the disease is known to be present in immigrant Asiatics.

The specific snails and fish prevalent in the waters of districts in which the disease is endemic do not inhabit the waters of the Pacific Slope, but representatives of the same families are present. Attempts to develop the larva of the fluke in these animals have thus far been negative.

SUMARIO

La clonorquiasis es una affección crónica, probablemente comunicable, y clasificada bajo las leyes de inmigración como enfermedad contagiosa y de peligro. Todos los extrangeros atacados de esta enfermedad son mandatoriamente excluíbles. La enfermedad existe y ha existido entre los nativos orientales de este país desde hace por lo menos veinte años. Prevalece en aquella región de la China que surte de emigrantes chinos a los Estados Unidos y también se observa, en número bastante perceptible, entre los chinos que solicitan admisión a los Estados Unidos.

No hay datos acerca de la infestación del hombre o de los animales que han residido continuamente en el país. Nuestra inspección en California de 407 personas, 38 perros, 8 gatos y 1468 cerdos indica que no hay infestación a pesar del hecho de que se sabe que existe entre los inmigrantes Asiaticos residentes. Los caracoles y los peces específicos que prevalecen en los lugares de endemicidad, no habitan las aguas de la falda del Pacífico aunque sí se observan ciertos representantes de la misma familia. No se ha podido conseguir que las larvas de las lombrices se desarrollen en estos animales.

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RESULTS OF TREATMENT FOR CLONORCHIASIS[1]

PRELIMINARY REPORT

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INTRODUCTION

I have recently reported the recognition in Boston of a considerable number of cases of clonorchiasis (1) (or clonorchiosis), most of them in Chinese examined at the United States Immigration Station in East Boston. Through special permission from Washington a few of the patients were landed for treatment at the Boston City Hospital.

None of the cases observed at the Immigration Station or at the Hospital showed any sign of disease other than a slight eosinophilia which was pretty constantly present but which might have been due to the coincident presence of other intestinal parasites which were found in all the cases of clonorchiasis observed at the Hospital.

Clonorchiasis is generally regarded as incurable but the fact that the use of intravenous injections of tartar emetic (tartrate of potassium and antimony) has led to extraordinary success in the treatment of all forms of schistosomiasis, leads to the reasonable hope that this drug might prove useful against other diseases caused by flukes. Moreover, a single severe case of clonorchiasis has already been reported by Brug (2), as having been cured by means of a course of intravenous injections of tartar emetic. I have seen no other record of the use of tartar emetic for clonorchiasis and know of no other case successfully treated by this or other means.

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[1] Read by title at nineteenth annual meeting of American Society of Tropical Medicine, San Francisco, Calif, June 25, 26, 1923.

At the Boston City Hospital we have had 3 cases under treatment, for which the data are now available, and we shall be prepared later to report on 3 more cases which are still under observation.

In all of these cases ova of *Clonorchis* were so few as to be difficult to find except in specimens of feces in which they had been concentrated by repeated centrifugalization and washing. The laboratory reports for ova in the case-records all refer to specimens so prepared.

Case 1 was treated with intravenous injections of tartar emetic and received no other treatment. Case 2 had tartar emetic followed by a few injections of arsphenamine, and case 3 had tartar emetic and one injection of arsphenamine.

The modes of preparation, of administration, and the dosage of tartar emetic advised by various authors differ considerably not only as between the several diseases for which the drug has been used extensively, but also as to its use in schistosomiasis.

Dr. Reid Hunt, of the Harvard Medical School, recently informed me that the "exact constitution" of tartar emetic is uncertain, that "what happens when solutions stand or are heated too long is not surely known," and that "under these circumstances attempts to prepare stable solutions are purely empirical."

PREPARATION OF TARTAR EMETIC SOLUTION

Castellani and Chalmers (3), under the head of kala-azar, say that a 1 per cent solution in warm normal saline solution should be used and that it should be sterilized "in flowing steam on two or three consecutive days" because if placed in the autoclave the drug is liable *to decompose and then to cause serious symptoms.* Some authorities, they say, advise using a solution merely filtered through a Chamberland filter. They have used a solution containing 0.5 per cent of carbolic acid which renders the solution unsuitable for use intravenously but such a solution may be administered intramuscularly in spite of its severely irritating properties which have led to the more general use of the drug by the intravenous route.

Manson-Bahr (4), under the head of oriental sore, says that solutions of antimony do not keep well, recommends that they be prepared with *freshly* distilled water, and states that they should not be boiled; but this last refers particularly to the organic compounds.

Contrary to Castellani and Chalmers, Cawston (5), in an article on bilharziasis, says that *toxic effects are less common* when using a solution of the drug *which has not been freshly prepared* and he believes that it may deteriorate within a few hours after boiling. He therefore dissolved the tartar emetic by boiling in distilled water and injected it before it had become cold.

For the treatment of bilharziasis, Christopherson (6), recommended a solution of ½ grain of tartar emetic in 20 mm. of distilled water to be further diluted with salt solution before injecting. To the initial dose of ½ grain was to be added 40 or 50 minims of normal salt solution. Injections were made with a 10 cc. syringe. More recently (7), he says "antimony tartrate is an irritant, and a strong solution must not be injected into the veins. Half a grain of antimony tartrate diluted with 3 cc. normal saline solution, is the strength I use; 2½ grains should be given with 10 cc. and intermediate quantities in proportion." "It is not necessary nor convenient," he adds, however, "to use a larger syringe than a 10 cc. Record. The needle should be of hypodermic thickness and should be sharp 1¼ inches in length, and a platino-iridium needle is best." He says that tartar emetic solution is stable and can be boiled as often as necessary.

Baujean (8), used a solution containing 0.02 gram to 1 mil. of normal saline (2 per cent). This he prepared according to need in quantitites of from 30 to 60 mils in a flask and sterilized it in the autoclave at 120°C. He injected the solution without dilution and had good results in both forms of bilharziasis. He emphasizes the importance of using a pure drug and he says that even this strong solution *if prepared in water instead of salt solution* has too low a titer to prevent hemolysis.

Taylor (9), used a solution containing 0.065 gram (1 grain) to 6 mils of freshly distilled water (1 per cent) and autoclaved it for one hour.

Dr. Alexander Randall of Philadelphia, who used tartar emetic for the treatment of inguinal granuloma, has kindly informed me by letter that at first he had a 1 per cent solution (about 1 grain or 0.065 gram in 6.5 mils) prepared *in distilled* water, put up in ampules and sterilized in the autoclave. Later he had stock solutions prepared in bulk, put up in flasks, and again sterilized the solution by autoclave before using. Since then he has had measured amounts of the drug put up in papers by the pharmacist and these have been dissolved as needed in normal saline solution and subsequently autoclaved. The same therapeutic effect was obtained by all three methods, but irritative effects appeared to be less when normal saline rather than distilled water was used.

From this conflicting testimony the following conclusions seem warranted:

1. A 1 per cent solution containing about 0.065 gram (1 grain) of the drug in about 6 mils of fluid is a suitable strength for injection.
2. When the drug is prepared in concentrated solution in distilled water it has generally been diluted with salt solution before use.
3. If originally prepared in 1 per cent solution, normal salt solution as the solvent is probably preferable to distilled water.
4. It is important to use a pure drug and to dissolve it in a pure solvent. It is not apparent why *fresh* distilled water should be specified unless because more likely to be pure.
5. There is no clear evidence of deterioration or of change in solutions of tartar emetic when sterilized and preserved under aseptic conditions in flasks, ampules or vaccine bottles.
6. Although Christopherson recommends sterilization by boiling, the experience of Randall, of Baujean and of Taylor, indicates that solutions of tartar emetic may safely be autoclaved.

At first we used a solution of 0.032 gram ($\frac{1}{2}$ grain) in 1 mil of distilled water and further diluted small doses of the tartar emetic with 5 mils and larger doses with 6 to 7 mils of normal salt solution. Injections were given with a 10 cc. syringe.

Later we tried salt solution instead of distilled water for the stock solution but observed a slight turbidity and a slight white precipitate on standing which led us to return to the use of distilled water for the stock solution. Very recently Mr. Walter G. O. Christiansen of the Department of Pharmacology of the Harvard Medical School, by analysis of the tartar emetic in use revealed the fact that the turbidity was caused by the presence of a small amount of oxide of antimony which is insoluble in water. Samples of tartar emetic obtained from a number of different druggists and pharmaceutical houses were then found to contain small amounts of lead, arsenic or iron as well as oxide of antimony.[2]

Solutions prepared from tartar emetic which had been purified in the laboratory were perfectly clear and Mr. Christiansen was unable to find any evidence of change after autoclaving them at a much higher temperature and for a longer period than is required for sterilization. The solutions showed no sign of change of any kind after keeping for four months. This work of Mr. Christiansen is to be published in the Journal of Laboratory and Clinical Medicine, Ann Arbor, Michigan.

We believe, therefore, that the clinician who uses tartar emetic intravenously should insist on obtaining a preparation from which a perfectly clear solution can be made, and which will conform in all respects to the U. S. P. standard. He may then make stock solutions which can safely be autoclaved and which will probably keep indefinitely if contamination be excluded.

DOSAGE AND ADMINISTRATION OF TARTAR EMETIC

Christopherson (10), first used $\frac{1}{2}$ grain (0.032 gram) every other day working up to 2 grains (0.13 gram). The course of treatment lasting from fifteen to thirty days. Subsequently, he gave the first few injections daily and, occasionally, increased the maximum dose to $2\frac{1}{2}$ grains (0.162 gram) (11). His total dosage was from 20 to 30 grains or (1.30 to 1.95 gram).

Low and Newham (12), followed Christopherson's dosage as did Baujean (8) except that his total did not exceed 20 grains over a period of nineteen days.

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[2] A sample of tartar emetic received from Charles Pfizer & Co., Inc., New York. conformed to the U. S. P. standard and was the best examined.

Day (13), found the minimal effective dose to be 13 grains and adds that a dose of 25 to 30 grains is not always enough.

Taylor (9), used from 1 to 2 grains on alternate days and later gave 3 grains twice a week.

The smallest doses and lowest total giving effective action were those of Cawston (5) (14) working in South Africa. He began with $\frac{1}{4}$ grain, gave $\frac{1}{2}$ grain on the second day and $\frac{3}{4}$ grain on the third, thereafter used from 1 to $1\frac{1}{2}$ grains on alternate days and found a total of 12 grains given over the period of one month was generally sufficient. The duration of treatment varied from three to five weeks.

Much larger dosage has been used for inguinal granuloma, for kala-azar, and for trypanosomiasis.

Injections have generally been given with a 10 cc. syringe and a fine needle. The injection should be given slowly and it is well that the solution should be warm.

UNTOWARD EFFECTS OF TARTAR EMETIC

Cushny (15), describes the toxic effects of antimony as follows: Small quantities cause perspiration, larger quantities nausea and vomiting. In acute poisoning there is violent and continued vomiting, profuse diarrhea, great weakness and collapse. The pulse is accelerated, at first, but becomes slow and irregular. The symptoms are due partly to the effects of emesis. When injected into the vein of an animal, antimony acts directly on the cardiac muscle. In fatal poisoning there is marked congestion and edema of the lungs. The mammalian heart is injured by the potassium when potassium chloride is administered intravenously.

The symptoms of chronic poisoning are deafness, headache, giddiness, confusion, drowsiness, indistinct sight, loss of appetite, general weakness and exhaustion with profuse diarrhea and rapid loss of weight.

Like other heavy metals says Cushny (15), antimony irritates the kidneys. It passes into the tissues much more slowly than arsenic and it is found in considerable quantities in the liver where it is stored for a time.

Low (12), and others believe that large doses of tartar emetic may, at times, cause fatty degeneration of the liver but Christopherson (6), in 1919 said that this is yet to be proved. He thought the fatty changes found at autopsy might have been due to other causes.

It is generally believed that tartar emetic is a powerful cardiac depressant. Much of the drug is eliminated through the kidneys and by the bowel.

Considering the very large number of cases of bilharziasis, kala-azar and other debilitating diseases in which tartar emetic has been used in recent years, the number of deaths which have been attributed to the drug is small. Toxic effects of a mild character generally give ample warning of danger when the limit of toleration has been exceeded. Cough, pharyngeal irritation and a feeling of constriction in the chest are commonly mentioned as coming on during or immediately after administration of the drug. Nausea or vomiting, syncope, rigor with fever, or sweating and collapse may supervene.

Cawston (5), observed severe effects in only one case of idiosyncrasy. There was great abdominal pain, vomiting and collapse about four hours after administration of $\frac{1}{4}$ grain of the drug to a patient of fifteen years of age. In another case he observed blackening of the skin under a wrist watch after a total of 12 grains of the drug had been administered.

Other effects observed are slight diarrhea, headache, giddiness, pruritus, bodily pain, stiffness of neck and shoulder muscles, loss of weight, induration at the point of injection, and, rarely, high fever. Tachycardia has been attributed to the treatment, but Christopherson (16), is convinced that in Egypt it is commonly due to other causes and is not to be attributed to tartar emetic.

Pain and stiffness about the shoulders were repeatedly observed by Randall (17), when using tartar emetic for inguinal granuloma.

Knowles (18), reported several deaths attributed to the use of antimony in kala-azar and Archibald and Innes (19), reported a fatal case of bilharziasis which they attributed to the same cause but which Christopherson (20), believes died of influenza pneumonia.

In the 1000 cases reported by Lasbrey and Coleman (21), there were but 10 deaths which could in any way be attributed to the tartar emetic. Six deaths occurred in the first 370 cases and only 4 in the remaining 630 cases. This lessened mortality they attributed to greater care in excluding patients exhibiting marked intolerance for the drug, to more gradual administration and to great moderation of dosage in very feeble subjects. No deaths took place immediately after an injection and usually some days supervened between the last injection and the fatality. The total amount of tartar emetic administered in some of these cases, they say, was very small. The usual symptoms preceding death in their cases were total loss of appetite, extreme debility and complete apathy. A sudden effort, such as rising from bed, was followed by fatal syncope. (Had sufficient expert nursing been available, some of these cases might have lived.)

Coincident with improvement in symptoms, patients may gain weight while under treatment, but Summons and Irving (22), observed that their patients felt poorly and lost weight (doses 0.03 to 0.12 gram = $\frac{1}{2}$ to 2 grains) and that larger doses always produced vomiting, headache and pain in the limbs.

PRECAUTIONS TO BE OBSERVED

Christopherson (11), says that tartar emetic in the dosage recommended by him for bilharziasis is not dangerous if used with care and judgment but he has again and again emphasized the fact that intercurrent disease of important organs such as heart, liver, kidneys and lungs should be looked for, and when these are present, tartar emetic should be used with the greatest caution. Bilharziasis patients, he says, are not usually feeble and emaciated as they become in the later stages of kala-azar, but feebleness or emaciation, when present, require that tartar emetic be used with great caution. He recommends that the tartar emetic be administered two or three hours after a meal but not after fasting.

According to Low and Newham (12), the patient should remain in bed the day of the injection and general constitutional or gastric symptoms should be carefully watched for. If they are severe and recurring, the treatment may have to be abandoned.

Albumin in the urine, they say, does not contraindicate treatment when it results from bilharziasis rather than from nephritis.

Day (13), has reported on 1000 cases successfully treated as outpatients. He gave the usual dosage daily for the first week and on alternate days in the second and third weeks. However, he exercised great care in the selection of the cases.

Cawston (14), believes that in a warm climate one is justified in treating most cases of bilharzia infestation as outpatients provided they are warned against the "very serious risk of contracting a 'cold' after injection, and provided they are within easy reach should any untoward symptoms such as throbbing in the head, vomiting, and faintness occur." It is unnecessary, he thinks, for adults to remain in bed if they will stay quietly in-doors for the rest of the day after an injection.

RESULTS IN SCHISTOSOMIASIS

Tartar emetic has been advocated as a specific for bilharzia by Christopherson. The drug has been used most extensively against *Schistosoma hematobium* but also in many cases of infestation with *Schistosoma mansoni* and in a comparatively small number of cases harboring *Schistosoma japonicum*.

In uncomplicated cases symptoms have nearly always subsided rapidly. Failures have generally been due to inability to pursue the treatment or to insufficient dosage from some other cause. Many cases having secondary complications have been benefited.

Deaths have been referred to above. They should be fewer in the future than in the past, for the dangers are better known than formerly.

RESULTS IN CLONORCHIASIS

The case reports appended show the dosage and dates on which medication was given. The stool examinations are listed, as well, in order to correlate the results.

In case 1 a total dose of 19½ grains of tartar emetic was given in fifty-two days, but after 8 grains had been administered in fifteen days ova were not again found in the feces. The treatment was continued, however, in order to increase the likelihood of a complete cure.

Practically the same total dose of tartar emetic was administered in case 2 as in case 1, but over the much longer period of ninety-seven days owing to repeated signs of intolerance, which several times rendered suspension of treatment necessary and its resumption with smaller doses. Meanwhile, the discharge of ova in the feces seemed to increase. Then, at the suggestion of Dr. Dario Guiterrez, who was my House-Officer at that time, and without much hope of favorable results, I prescribed a course of injections of arsphenamine. After 3 doses of arsphenamine totalling 1.1 grams had been given ova were not again found.

It is a question whether or not ova would have disappeared in case 2 after the use of arsphenamine had not tartar emetic been used first.

Cases 1 and 2 were held under observation for a further period of a month or more at the Immigration Station in East Boston. The ova of *Clonorchis* were looked for repeatedly there but were not found. Both patients were ultimately released as cured because no evidence of disease remained.

Whether or not these apparent cures will be permanent depends on whether or not the treatment killed all the flukes. It is conceivable, on the one hand, that the flukes were killed in the early stages of treatment and that eggs deposited previously continued for some time to be discharged, or, on the other hand, that the treatment did not kill the flukes but merely caused them to cease for a considerable period of time to deposit eggs.

The outcome in the third case was very disappointing. Although this patient was of much lighter build than either of the others he received about 50 per cent more tartar emetic than either of them and had it all within the much shorter period of forty-four days as contrasted with fifty-two days in case 1 and ninety-seven days in case 2. Owing to circumstances stated in the report of the case, treatment with arsphenamine, after the course of tartar emetic, was discontinued before a conclusion as to its possible effect could be reached. This case, therefore, cannot be considered as a failure for combined treatment, but it was a failure for tartar emetic alone.

VARIOUS POINTS OF INTEREST

During and after the courses of treatment with tartar emetic the ova found in the stools were carefully scrutinized for evidences of action upon them by the drug. In schistosomiasis, while treatment is in progress, the ova passed become blackened and shrivelled and fail to hatch when the urine is diluted with water.

No change was observed in the *Clonorchis* ova and hatching experiments were not tried because the ova of *Clonorchis* probably do not hatch in the water. These ova seem to be less permeable to water than are those of *Schistosoma.* Similarly, they may be less permeable to tartar emetic.

In case 2 and 3 very slightly lower eosinophile counts were obtained during treatment than before treatment. Case 1 showed a slight spontaneous difference suggesting that the reduction in the other cases may have had no relation to treatment. Christopherson's (6), observations seem to show that the eosinophilia may be increased by a course of treatment with tartar emetic.

The liver function test performed in case 3 before treatment was begun, failed to show evidence of derangement of the liver. It seems probable, however, that the liver may sometimes function normally even when diseased. Moreover, experience with this new test is too limited to make possible satisfactory interpretation of results.

The examination of bile for ova in case 1 was performed because Dr. Chester Jones of the Massachusetts General Hospital last year found ova in bile which he withdrew from the gall bladder in a case of vesicle schistosomiasis.

It was thought wise to allow an interval after the course of treatment with tartar emetic before administering arsphenamine and to begin with a small dose of the latter.

The interval in case 2 was sixteen days and case 3, seven days, and the initial dose of arsphenamine in each case was 0.3 gram.

SUMMARY

The reasons for trying tartar emetic in clonorchiasis, the various methods of preparation of the drug, dosage, administration, the untoward effects, precautions to be observed and the results obtained by others in the treatment of schistosomiasis have been dealth with.

Application of the treatment to three cases of clonorchiasis has been described and full reports of the cases are appended.

Treatment with tartar emetic alone appears to have been completely successful in 1 case of clonorchiasis and to have failed in 2 others. One of these cases was apparently cured by a course of tartar emetic followed by a few small doses of arsphenamine. Trial of this method in the third case was unavoidably interrupted.

No change in the appearance of the ova was observed. They did not shrivel or turn black as do the ova of the *Schistosoma* when tartar emetic is used.

Other points of interest in connection with the cases have been touched upon.

CONCLUSIONS

The cases being few, final deductions cannot be drawn However, it may be said, on the one hand, that results so far obtained have not been as satisfactory as they usually are after the use of tartar emetic in schistosomiasis, and, on the other hand, that there is ground for making further trial of the methods described above.

SUMARIO

Se hace un estudio en este artículo acerca de del uso del tártaro emético en la clonorquiasis, los varios métodos de preparación de la droga, su dosis, administración, efectos y precauciones que se deben observar, asi como los resultados obtenidos por otros en el tratamiento de la esquistosomiasis.

Se explica en detalle el tratamiento que se siguió en tres casos de clonorquiasis y se dán todos los datos referentes a su historia clinica.

El tratamiento con tártaro emético tuvo éxito en uno de los casos de clonorquiasis pero resultó inútil en los dos restantes. Se consiguió la curación completa de uno de ellos después de haberse proseguido el tratamiento con varias dosis pequeñas de arsenofenamina. Debido a una interrupción inevitable no se pudo volver a ensayar esta combinación de drogas en nuestro tercer caso.

No se notó ningún cambio en la apariencia de los huevos ni tampoco el encogimiento y ennegrecimiento que les ocurre a los huevos del esquistosoma después de la administración del tártaro emético.

Se someten varios otros aspectos interesantes de la cuestión.

APPENDIX

CASE 1

W. Y., Chinese, male, aged fifty-one. Admitted for treatment July 21, 1922, from Immigration Station with diagnosis of clonorchiasis

History. Unimportant.

Physical examination. Well developed and rather stout. Unusually large for a Chinaman. Weight 160 pounds. Otherwise negative.

Laboratory examinations

Urine: Negative throughout stay in hospital.

Blood:
- June 26: Hemoglobin (Talquist) 80 per cent. Eosinophiles 4 per cent.
- July 19: Hemoglobin (Talquist) 80 per cent. Eosinophiles 2 per cent.
- July 24: Hemoglobin (Talquist) 95 per cent.

Feces:
- July 10: Ova of Clonorchis +.
- July 30: Ova of Clonorchis +.

Tartar emetic begun July 31:
- August 14: Ova of Clonorchis not seen.
- August 22: Ova of Clonorchis not seen.
- August 28: Ova of Clonorchis not seen.
- August 31: Ova of Clonorchis not seen.
- September 4: Ova of Clonorchis not seen.

September 7: Ova of Clonorchis not seen.

September 19: Ova of Clonorchis not seen.

Ova of *Ascaris* and of *Trichiuris* were found repeatedly.

Bile:

Obtained with the duodenal tube from the gall bladder on July 30 showed no ova of *Clonorchis.*

Doses of tartar emetic

July 31	¼ grain	August 20	1 grain
August 2	½ grain	August 22	1 grain
August 3	½ grain	August 26	½ grain
August 4	¾ grain	August 29	½ grain
August 6	1 grain	August 31	½ grain
August 7	1 grain	September 8	1 grain
August 8	1 grain	September 10	1 grain
August 10	1½ grains	September 13	1 grain
August 13	1½ grains	September 15	1 grain
August 15	1½ grains	September 18	½ grain
August 18	1 grain	September 21	1 grain

Ova not found on and after August 14.

Total dose 19½ grains (1.27 grams) in fifty-two days.

Progress of case

The treatment was begun on July 31 and ended on September 21, a period of fifty-two days. During this time a total dose of 19½ grains of tartar emetic was administered. Although the patient was rather large and vigorous for a Chinaman, and weighed 160 pounds, he exhibited intolerance to the larger doses so that treatment was prolonged by the necessity of using small doses of tartar emetic.

Signs of the toxic action of tartar emetic appeared on the night following the first dose of 1½ grains given on August 10 and consisted of burning pain and stiffness of the right shoulder (the injection had been given in the left arm). The same dosage was administered twice more on alternate days, but the pain continuing, the dose was reduced to 1 grain on August 18. On August 18 the patient complained of pain and stiffness of both shoulders and neck which began about ten hours after the last injection. He felt otherwise well. Sodium-bicarbonate was prescribed on August 23 in dose of 2 ounces daily. On August 26 the dose of tartar emetic was reduced to ½ grain on account of persistence of the symptoms above described and on the following day pain was very slight. During the first week of September medication was discontinued. On September 5 the patient said the pain had entirely disappeared. On September 8 doses of 1 grain were again started.

The only other untoward symptom was pain in the right arm where an intravenous injection had been given. There had been swelling and slight but persistent pain in the region of the vein for some time until September 22 when a flaxseed poultice was applied. Within a few days thereafter, the lesion suppurated and on September 29 about 7 cc. of pus were evacuated by incision. Prompt healing followed. The abscess was probably caused by slight leakage of the tartar emetic solution into the tissues.

Eight consecutive examinations of the feces between August 14 and October 1, a period of six weeks, had shown no ova of *Clonorchis*. Accordingly, the patient was discharged on October 2 to the Immigration Station where he was kept under observation for a month and where several more examinations of the feces were made with negative results.

No evidence of the persistence of the disease having been found, this patient was then released.

CASE 2

H. M. Y., Chinese, male, aged thirty-eight. Admitted for treatment August 2, 1922, from Immigration Station with diagnosis of clonorchiasis.

History. Unimportant.

Physical examination. Well developed and nourished. Large and strong looking for a Chinaman. Otherwise negative.

Laboratory examinations

Urine: Negative throughout stay in hospital.

Blood:

June 22: Hemoglobin 90 per cent. Eosinophilia 8 per cent.
November 10: Red cells 4,920,000. Eosinophiles 1 per cent.

Feces:

June 23: Ova of *Clonorchis* +.
June 27: Ova of *Clonorchis* +.
June 29: Ova of *Clonorchis* +.

Tartar emetic begun August 2:
- September 6: Ova of *Clonorchis* +.
- September 19: Ova of *Clonorchis* +.
- September 28: Ova of *Clonorchis* ++.

Arsphenamine begun October 21:
- October 7: Ova of *Clonorchis* ++.
- October 22: Ova of *Clonorchis* not seen.
- October 29: Ova of *Clonorchis* +.
- November 6: Ova of *Clonorchis* not seen.
- November 8: Ova of *Clonorchis* not seen.
- November 13: Ova of *Clonorchis* not seen.
- November 16: Ova of *Clonorchis* not seen.
- November 20: Ova of *Clonorchis* not seen.

Ova of *Ascaris* and of *Trichiuris* were found repeatedly.

Doses of tartar emetic

August	2	$\frac{1}{4}$ grain	September	4	1 grain
August	3	$\frac{1}{2}$ grain	September	8	1 grain
August	4	$\frac{1}{2}$ grain	September	10	1 grain
August	10	$\frac{1}{6}$ grain	September	13	1 grain
August	11	$\frac{1}{2}$ grain	September	15	1 grain
August	13	$\frac{3}{4}$ grain	September	18	1 grain
August	20	$\frac{1}{2}$ grain	September	21	$1\frac{1}{2}$ grains
August	22	$\frac{1}{2}$ grain	September	27	$1\frac{1}{2}$ grains
August	26	$\frac{1}{2}$ grain	September	29	$1\frac{1}{2}$ grains
August	28	$\frac{1}{2}$ grain	October	2	$2\frac{1}{4}$ grains
August	29	$\frac{1}{2}$ grain	October	5	$1\frac{1}{2}$ grains
September	1	$\frac{1}{2}$ grain			

Total dose 20 grains (1.3 grams) in ninety-seven days.

Doses of arsphenamine

October	21	0.3 gram
October	28	0.4 gram
November	4	0.4 gram

Ova not found on or after November 6.

Total dose 1.1 gram (17 grains) in fourteen days.

Progress of case

On the evening of August 2, $\frac{1}{4}$ grain of tartar emetic having been administered in the morning, the patient noticed an eruption on the face and neck and complained of itching in the same region. Examination on the following morning showed a diffuse erythematous eruption associated with slight swelling of the skin and with numerous small vesicles on the face, neck, and shoulders. The patient felt otherwise well.

Although the eruption was thought to be caused by the drug, additional ½ grain doses were administered on the next two days. Then, because the eruption tended to increase, medication was discontinued for six days.

Treatment was resumed on August 10 beginning with only ⅙ grain of the drug. On the following day ½ grain was administered and on the second day thereafter a dose of ¾ grain. About midnight of that date the patient noticed a new eruption on the abdomen and extremities. The rash consisted of numerous small red papules. Most of them disappeared entirely on pressure but some of them retained a little color. Desquamation followed. Coincident with the appearance of this eruption the face had a congested and slightly swollen appearance and there was slight edema of the legs. The urine remained free from albumin. The tartar emetic was suspended for seven days and sodium bicarbonate was prescribed in the dose of 3 ounces daily. By August 18 the eruption had entirely disappeared but very slight edema of the legs persisted.

On August 20 treatment with tartar emetic was resumed using ½ grain doses and the sodium bicarbonate was continued. No further symptoms having arisen, dosage was increased on September 1 to 1 grain; on September 21 to 1½ grains; and on October 2 to 2¼ grains. The drug was administered during this period every second or third day.

The 2¼ grain dose was given in the afternoon. The patient vomited his supper eaten at 5 p.m., there was pain near the site of the injection and an erythematous eruption appeared on the flexor surfaces of the elbows and forearms. This eruption having disappeared, a final dose of 1½ grains was given on October 5. On the following morning there was a pronounced erythema associated with many minute vesicles on the arms. A total of 20 grains of tartar emetic had been given between July 2 and October 5, a period of ninety-seven days. A few days later there was evidence of slight inflammation around the vein at the right elbow. After poulticing for a few days the inflammation subsided leaving behind what felt like a thickened and thrombose vein.

The ova of *Clonorchis* not only had not decreased, but seemed more numerous than before. No modification of their appearance (such as occurs in *Bilharzia* ova in the course of treatment with tartar emetic) could be discovered.

A course of treatment with arsphenamine was started on October 21. Three doses were given at intervals of one week. Meanwhile, ova rapidly became less numerous and between November 6 and 20 inclusive, five consecutive examinations were negative.

The patient was discharged to the Immigration Station on November 20 where he was kept under observation for more than a month. Repeated examinations for ova were made there, and specimens were sent to the Public Health Laboratory in Washington, but ova were not again found. The patient was finally discharged.

CASE 3

L. B. C., Chinese, male, aged thirty-two. Admitted July 12, 1922, from Immigration Station with diagnosis of clonorchiasis.

History Unimportant.

Physical examination. Well developed. Tall and rather slender. Weight 115 pounds.

Laboratory examinations

Urine: Negative throughout stay in hospital.

Blood:
- July 19. Hemoglobin 85 per cent. Eosinophiles 5 per cent.
- October 2. Red cells: 5,200,000.
- November—. Eosinophiles 1 per cent.

Feces:
- July 14. *Clonorchis* ova not seen.
- July 17. Ova of *Clonorchis* +.
- August 22. Ova of *Clonorchis* +.
- August 31. Ova of *Clonorchis* ++.
- September 19. Ova of *Clonorchis* ++.
- September 28. Ova of *Clonorchis* ++.
- October 20. Ova of *Clonorchis* not seen.

Tartar emetic begun October 27:
- October 29. Ova of *Clonorchis* +.
- November 6. Ova of *Clonorchis* +.
- November 8. Ova of *Clonorchis* +.
- November 13. Ova of *Clonorchis* not seen.
- November 16. Ova of *Clonorchis* +.
- November 20. Ova of *Clonorchis* not seen.
- November 27. Ova of *Clonorchis* ++.

December 1. Ova of *Clonorchis* +.
December 7. Ova of *Clonorchis* not seen.
December 12. Ova of *Clonorchis* +.
December 14. Ova of *Clonorchis* not seen.

Arsphenamine given December 18.

Ova of *Ascaris* and of *Trichiuris* were found repeatedly.

Liver function test. On September 8, 1922, Dr. S. M. Rosenthal kindly performed his test of liver function injecting 5 mgm. per kilo of body weight (260 mgm.) of phenoltetrachlorphthalein. The result was a normal curve showing no evidence of derangement of liver function.

Electrocardiograms. Taken by Dr. W. D. Reid before and after an experimental dose of ½ grain of tartar emetic administered intravenously on September 1. A slight difference was observed which Dr. Reid thought might perhaps have been caused by the drug.

Doses of tartar emetic

September 1. 0.5 grain for electrocardiogram.

October 27	½ grain	November 18	1½ grains
October 29	½ grain	November 20	1½ grains
October 31	¾ grain	November 23	1½ grains
November 2	1 grain	November 25	1½ grains
November 4	1 grain	November 27	2 grains
November 6	1 grain	November 29	2 grains
November 8	1 grain	December 1	2 grains
November 10	1 grain	December 3	2 grains
November 12	1½ grains	December 5	2 grains
November 14	1½ grains	December 9	2 grains
		December 11	2 grains

Total dose 30¼ grains in forty-four days.

Arsphenamine. December 18, 0.3 gram.

Progress of case

This patient never had either pain in the neck and shoulders, any eruption, nausea or vomiting so that it was not necessary at any time to interrupt the treatment.

Immediately after receiving the first dose, however, the patient had a transient sensation of constriction in the throat.

Although ova of Clonorchis were perhaps less numerous toward the end of the treatment with tartar emetic, no striking change occurred and the appearance of the ova was not altered. A total of 30¼ grains had been given from October 27 to December 11, a period of forty-four days.

A course of arsphenamine was then begun but after the first dose had been given, word was received that the patient must be deported.

I then learned with regret that treatment had been applied under a misapprehension and that the necessary official authority for it had not been granted.

The patient was discharged December 21, 1922.

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A SERIES OF CASES GIVING A POSITIVE WEIL-FELIX REACTION[1]

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The Weil-Felix reaction has received general acceptance as an empiric laboratory test of considerable merit in establishing the diagnosis of typhus fever. The testimony of writers is uniform in affirming that the reaction is positive in over 90 per cent of cases of clinical typhus. It may appear as early as the fourth day of the disease, but usually does not develop until the second week and yields maximal titres about the time that the temperature returns to normal or shortly thereafter. Complete agglutination of the strain of *B. proteus* known as X_{19} in a dilution of the patient's serum greater than 1:50 is looked upon as of specific diagnostic significance. So far as can be determined the organism has no etiologic relationship to the disease and the mechanism of the reaction is not clearly understood. An excellent discussion of the immunological principles involved and the technique of the reaction has been published by Bengtson in the Public Health Reports, October 31, 1919.

In spite of this fact, the evidence previously available has indicated a high degree of specificity for the reaction. Weil and Felix (2) examining the sera of 632 patients not having typhus, including cases of typhoid, meningitis, variola and relapsing fever, found that 12 per cent gave positive reactions in a dilution of 1:25 but not higher, and in practically all cases the agglutination was incomplete and delayed. Sacquépée and Delavergne (3) reported the reaction negative in 14 cases other than typhus. In Fairley's series in Egypt, 120 such cases were negative in a dilution of 1:20. Montefusco (5) reported the reaction negative in a large number of cases other than typhus. Napier (6) concluded that 8 per cent of normals and 25 per cent of febrile cases other than typhus may give a titre as high as 1:50, but not higher. Holt-Harris and Grubbs (7), at Ellis Island, examined the sera of 737 cases of various acute diseases, 264 persons suffering from chronic diseases and 634 apparently normal persons. In their experience all cases giving a positive reaction have been undoubted typhus fever. Chambers (8) examining 370 sera from miscellaneous admission to the naval hospital, Washington, D.C., found only 1 positive and felt that a previous attack of typhus could not be entirely excluded in this case. Many others have recorded similar experience with the test, among them Kelly (9), who examined the blood of nine cases of Rocky Mountain spotted fever, only one case of which gave a positive Weil-Felix reaction, which could not be confirmed.

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From a review of the literature the impression is gained that the Weil-Felix reaction is seldom, if ever, positive in normal persons nor in those diseases other than typhus which have been investigated. The purpose of this paper is to report a series of cases occurring in Alabama which gave a positive Weil-Felix reaction. Clinically they resembled mild typhus or "Brill's Disease," but upon careful consideration of all the facts, clinical and epidemiological, considerable doubt arises as to their proper classification.

CASE REPORTS

In June 1922, Dr. W. W. McGehee of Montgomery, Ala., reported to the State Department of Health a case of suspected typhus. Investigation revealed the following facts:

Case 1. White, male, aged twenty-eight, waiter in cafeteria, residence Montgomery. Had not been outside city for six weeks before onset. Cleanly in habits; no louse infestation.

Onset June 5 with severe headache and fever; better the following day; then became worse and entered the hospital. Eruption appeared on the fourth or fifth day, dull red, maculo-papular, pin-head to pea-sized spots, some of which developed a purplish center which did not disappear on pressure, distributed over chest, back and extremities. Fever ranged about 101 to 102 and remained steadily elevated, coming down by lysis toward the end of the second week. He was never very sick; complained chiefly of headache and backache. During the second week there was a slight bronchial cough. He was apparently well on about the fourteenth day.

Blood culture: Negative, fifth day of disease.
Widal reaction: Negative, fifth and eighth day of disease.
Leucocyte count: 7000, fifth day of disease.

A specimen of blood taken from this patient on the fifth day of the disease was sent to the Hygienic Laboratory, Washington, D. C., for the Weil-Felix test. It was entirely negative in dilutions even as low as 1:25.

A second specimen of blood was taken on the eighth day and forwarded to the Hygienic Laboratory. Complete agglutination of culture X_{19} was obtained in a dilution of 1:200 and partial agglutination in a dilution of 1:800.

In view of the accepted specificity of the Weil-Felix reaction and its development during the course of a febrile illness with eruption, lasting about two weeks, a tentative diagnosis of typhus fever or Brill's Disease seemed justified, in spite of the fact that there was no evidence of louse infestation and no known source of infection.

Inquiries directed to physicians practicing in Montgomery elicited the fact that, so far as was known, typhus fever had not occurred in the city in recent years, although four cases with a clinical course similar to the one cited are said to have been observed. All of these had been persons in good circumstances with no evidence of louse infestation. Aside from these, no illnesses of a suspicious character were occurring among the poorer classes of the population. There is in this city no "foreign quarter" receiving immigrants from southern or eastern Europe, nor from Mexico, the two sources from which typhus is expected to originate.

Following the occurrence of this case of supposed typhus in Montgomery, the medical profession of this city, of Mobile and of Birmingham were called in special meetings and requested to be on the look out for cases of a similar nature. In the succeeding eight months, 13 cases of illness clinically similar have been investigated and in 11 of these the Weil-Felix reaction has been found positive. In the remaining 2, a blood specimen taken during the first week of the illness proved negative and it was impossible to procure a second specimen later in the course of the illness when it might have been expected to become positive. A brief history of the cases which showed a positive reaction is given below:

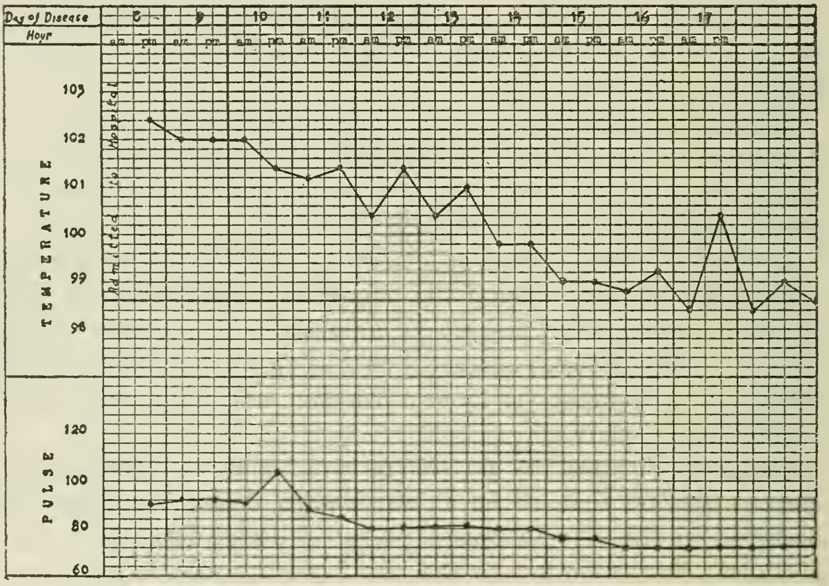

Chart 1. Course of the Temperature and Pulse from the Eighth Day of the Disease Onward in Case 2

Case 2.[2] Patient of Dr. J. H. Hope of Mobile, Alabama. White, female, aged sixty-two, living in good circumstances. No suspicious illness had previously occurred in the household. Had not been outside Mobile for several months. No evidence of louse infestation.

Began to feel badly on July 10. Definite onset with severe vomiting, diarrhea and fever on July 15.

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[2] We are indebted to Dr. C. A. Mohr, County Health Officer, and Surgeon W. H. Slaughter, U.S.P.H.S., for the investigation of cases 2 and 5.

Physician called on July 19 (fifth day), found her complaining of pains in the back of the head, in the back and aching in the knees; temperature 103; pulse 96; conjunctivæ injected; tongue heavily coated; chest negative except for feeling of "soreness"; abdomen slightly tympanitic; spleen slightly enlarged.

The eruption was already apparent as a few "rose spots" scattered over the abdomen; during the next days these spread over the entire body. The temperature reached its highest on the 21st and the patient entered a private hospital on the following day. The fever ran an uncomplicated course and came down by lysis to normal on about the fifteenth day.

Blood: Negative for malaria, July 19.

Widal reaction: Negative, July 19, 22, and 27.

Macroscopic agglutination for *B. typhosus*, para-A and para-B: Negative, July 27 (thirteenth day).

Weil-Felix: Positive (X_{19} in a dilution of 1:1200, July 27, thirteenth day).

Case 3. Patient of Dr. Scott of Headland, Alabama. White, female, aged thirty-two, had not been away from Headland for several weeks past; no history of louse infestation.

Onset July 24, with headache, pain in eyes and vague aches all over body; nausea and some vomiting, slight looseness of bowels. Temperature ranged from 99 to 104. "Rose spots" were on abdomen and arms and were still apparent on August 4, although the temperature was normal at this time (twelve days).

Blood culture for *B. typhosus:* Negative on eleventh day of illness.

Macroscopic agglutination for *B. typhosus*, para-A and para-B: Negative on eleventh day of illness.

Weil-Felix: Positive on eleventh day (X_{19} and X_2 both agglutinated in a dilution of 1:160).

Case 5. Patient of Dr. J. H. Dodson of Mobile. Colored (Mulatto), male, aged nineteen, single, laborer. Had not been outside city in several months; no louse infestation; no suspicious illness among family or co-laborers.

Onset July 25, abrupt with chilly sensations, fever, headache and pain in the back. On July 27, the physician called and found him with a temperature of 103, abdomen somewhat tympanitic; spleen enlarged. He seemed dull and apathetic. On July 31 (sixth day) eruption was first noted resembling somewhat a typhoid roseola; appeared on abdomen and chest, later extended well over limbs and trunk. Temperature was fairly continuous, ranging about 103 to 104. On August 5 (twelfth day of disease) it dropped abruptly and reached normal the following day. Generalized macular rash still visible on August 9.

Blood: Negative for malaria.

Widal: Negative, August 2, 3, 4, and 5.

Weil-Felix: Positive (X_{19} in a dilution of 1:640, August 3, tenth day; X_2 negative).

Two guinea-pigs inoculated intraperitoneally with blood of patient on thirteenth day of disease, failed to develop a febrile response.

Case 6. Patient of Drs. McGehee and Mount, of Montgomery. White, female, middle age, wife of lawyer. Had not been outside of city, family in good circumstances. No evidence of louse infestation.

Onset about October 8, sudden with severe headache, fever and general muscular pains. The physician made a diagnosis of dengue. The temperature was about 102 at first, but rose on the third day to 104. About the fourth or fifth day she developed an eruption—at first rather faint, scattered macules, particularly over chest and arms, later spreading all over the body, until she looked almost as if she had measles, though the color was a little darker red. The eruption was distinctly macular; it could not be felt. The eruption spread and became more pronounced during the first week, then waned and faded, so that about the third week it had disappeared. At its height it had a distinct purplish tinge. The most pronounced symptom was violent headache. This was so severe that a lumbar puncture was done—clear fluid under pressure with no pathology obtained. About the fifth day, she developed a broncho-pneumonia. At times she was flighty, delirious. Duration—about three weeks in all—temperature came down by crisis on about the twentieth day, from 105 to 96. Recovery uneventful.

White blood count: 4500 on the fourth day of the disease. During the second week the count was 5000 and then 6000. On the day of the crisis the count was 15,000. Differential count on the fourth day showed a slight increase in mononuclear cells.

Urine: Trace of albumin. No casts.

Malaria: Negative.

Weil-Felix: October 19, about the fourteenth day; positive in dilution of 1:640 with X_{19}.

Case 7. Patient of Dr. W. W. McGehee of Montgomery. White, male, aged fifty, owner of bottling works, gentleman in good circumstances. Had not been away from Montgomery for several months except short distances to hunt. No louse infestation.

Onset November 12, sudden with chill, severe headache, and nausea.

Rash appeared on fourth or fifth day—very fine macules, dull red in color, distributed over trunk and extremities. Temperature came down at the end of second week but on the sixteenth day there was a rise and some evidence of broncho-pneumonia with pleurisy at the right base. This lasted five to six days and thereafter his convalescence was uneventful.

White blood cell count: 4500.

Blood: Negative for malaria.

Macroscopic agglutination of *B. typhosus*, para-A and para-B typhosus: Negative on December 7 (twenty-fifth day).

Weil-Felix: Positive (X_{19} in a dilution of 1:320; X_2 in dilution of 1:40 on December 7, twenty-fifth day after onset).

Case 8. Patient of W. W. McGehee of Montgomery. White, female, aged thirty-eight, housewife in good circumstances. No evidence of louse infestation. Wife of case 7, and apparently secondary to his case. Onset about eight days after husband with acute gastrointestinal symptoms; later complained of severe pain over left lower abdomen; very little headache. Rash developed about the fourth day, maculo-papular in character, pin-head to pea size, distributed over chest and arms particularly. Temperature quite high—reached 104 at one time. Returned to normal about end of second week.

Macroscopic agglutination of *B. typhosus*, para-A and para-typhoid-B: Negative on December 7 (sixteenth day of disease).

Weil-Felix: Positive (X_{19} in dilution 1:80; X_2 in dilution 1:40 on December 7, sixteenth day of disease).

Case 9. Patient of Dr. W. W. McGehee of Montgomery. Male, white, aged thirty-five, works in pool room and restaurant. Cleanly in personal habits, no evidence of louse infestation. Had not been outside city for several months.

Sudden onset with chill, fever and headache about November 25. Rash appeared on fourth or fifth day, maculo-papular in character, distributed particularly over chest and arms. Slight cough during second week. Temperature returned to normal about twelfth day.

Macroscopic agglutination of *B. typhosus*, para-typhosus-A and para-typhosus-B: Negative on December 10 (fifteenth day of disease).

Weil-Felix: Negative on December 1 (fifth day of disease).

Weil-Felix: Positive on December 10 (fifteenth day of disease). (X_{19} in dilution of 1:1280; X_2 negative).

Case 10. Patient of Dr. W. W. McGehee of Montgomery. Female, white, aged sixty. Housewife of retired railroad conductor, living in good circumstances. No evidence of louse infestation. Had not been out of town.

Onset December 19, sudden with chill, fever and pain over right upper quadrant of abdomen (gall-bladder region); little or no tenderness on pressure. Rash appeared on fourth or fifth day, dull red maculopapules; irregular in size; chiefly over chest and arms. Somewhat drowsy and apathetic. Uncomplicated course and temperature reached normal the latter part of the second week.

White blood cell count: 4500, December 27.

Macroscopic agglutination for *B. typhosus*, para-typhosus-A and para-typhosus-B: Negative on December 27.

Weil-Felix: Positive (X_{19} showed partial agglutination 1:80; specimen taken on eighth day of disease).

Six guinea-pigs inoculated intraperitoneally with 1 to 3 cc. of patient's blood on eighth day of disease. All died within three days. No gross pathological lesions. Cultures of heart's blood in dextrose broth, aerobically and anaerobically, were negative.

Case 12. "H," patient of Dr. C. G. Laslie of Montgomery, Alabama. Works in stock yards. Male, white, aged thirty-five, living in Montgomery, Alabama. Has not been out of town recently. Living in cleanly surroundings; no history of louse infestation.

Complained of headache and not feeling well on February 13. On February 15 had severe headache, chills and high fever. Felt better the next morning and went to work (remission). Returned home that evening much worse and called physician. Patient was severely prostrated, "felt tired" and ached all over particularly severe frontal headache, some nausea and vomiting. Fever ranged from 102 to 103 and remained steadily elevated during first week. Eruption first noticed about the fifth day, dull red, pea-sized macules, most numerous over abdomen, but a few scattered over arms and back. Faded very quickly and was scarcely perceptible on the eighth day of the disease.

Fever came down gradually and reached normal about fourteenth day. Patient still suffered from prostration and depression.

Urine examination: Nothing abnormal except a few pus cells.

White blood cell count: 9000, fifth day of disease. Differential count: Polymorphonuclears, 51 per cent, on eighth day of illness; lymphocytes, 43 per cent; transitional type, 7 per cent.

Wassermann reaction: Negative.

Weil-Felix: Positive on February 21, sixth day of disease; X_{19}, 1:80; X_2, negative. Positive on February 26, twelfth day of disease; X_{19}, 1:640; X_2, negative.

Macroscopic agglutination of *B. typhosus*, para-A and B: Negative on February 21 (sixth day of disease). Negative on February 26 (twelfth day of disease).

Case 13. "B," patient of Dr. G. L. Wood, Andalusia, Alabama. Residence, Andalusia; occupation, barber. White, male, aged thirty-two; had not been out of town within six weeks; no history of louse infestation.

Onset sudden with chills, fever and headache about February 8. Had severe nausea and vomiting. Fever remained high with slight morning remission. Headache very severe; slightly delirious at times. *No eruption was noticed by physician or patient at any time.* The fever came down by lysis after about two weeks illness. Felt extremely weak and prostrated for some time after recovery.

Widal reaction: Negative on February 13 (about fifth day).

Specimen of blood: Four weeks after onset, (March 10, patient completely recovered) showed no agglutinating power for *B. typhosus*, B. para-A or B. para-B.

Weil-Felix: At this time, was positive with X_{19} in a dilution of 1:320.

In all but the first case the Weil-Felix reaction was performed in the laboratories of the Alabama State Board of Health. The cultures used were the X_{19} and X_2 strain of *B. proteus* (1) obtained from the Hygienic Laboratory through the courtesy of Surgeon G. W. McCoy. A freshly grown agar culture was used. The suspension was washed off with 5 to 7 cc. of normal salt solution and added in 0.5 cc. amounts to equal volumes of serum dilutions—1:10 to 1:1280—with the usual controls. The tests were incubated at 37°C. for two hours and the final reading made after standing over night—about fourteen hours—at room temperature. The titre represents the highest dilution in which definite clumping (fine flocculation) could be seen with the unaided eye.

In table 1 is shown a summary of the tests performed upon the ten cases cited. As negative controls during the period of this work, 34 specimens of serum from miscellaneous sources, specimens submitted for the Wassermann test, were examined. None of these showed agglutination of either strain in greater dilution than 1:40.

TABLE 1

Summary of the results of the Weil-Felix test on the series of cases reported

CASE NUMBER	FIRST WEEK OF DISEASE		SECOND WEEK OF DISEASE		THIRD OR FOURTH WEEK OF DISEASE	
	X_2	X_{19}	X_2	X_{19}	X_2	X_{19}
1	0	0	0	1:800	+	–
2	–	–	0	1:1280	–	–
3	–	–	1:160	1:160	–	–
5	–	–	0	1:640	–	–
6	–	–	0	1:640	–	–
7	–	–	–	–	1:40	1:320
8	–	–	–	–	1:40	1:160
9	0	0	–	–	0	1:1280
10	–	–	0	1:80*	–	–
12	0	0	0	1:640	–	–
13	–	–	–	–	–	1:320

* Eighth day of disease.

It is noteworthy that although the Weil-Felix reaction has been found negative in many diseases other than typhus, so far as has been ascertained, none of the observers have worked with dengue. Owing to the fact that this disease was prevailing at the time and entered into consideration in the differential diagnosis, a number of specimens[3] were obtained from persons who had had typical attacks of this disease, lasting three to seven days. Two of the specimens were taken during the course of the illness. The others were taken after recovery as follows: 1 specimen, three weeks after recovery; 2, six weeks; 2, eight weeks; 2, ten weeks; 3, twelve weeks; 1, thirteen weeks; 1, sixteen weeks. All of these specimens failed to agglutinate X_{19} in a dilution greater than 1:40.

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[3] We are indebted to Dr. C. C. Bass of Tulane University Medical School for several specimens.

DISCUSSION

An occasional case of "sporadic typhus" in an American city is not an unusual nor a particularly remarkable occurrence. At first there was little doubt but that the original diagnosis was the correct one. As the data of the whole series accumulated, many reasons appeared to increase the doubt. All, with one exception, occurred in persons of cleanly habits and good circumstances. None showed any evidence of louse infestation at the time of the illness nor could they give any history of louse bites. No source of infection with typhus could be demonstrated—no immigrant population that might serve as a possible reservoir of infection and no illnesses of a suspicious character occurring among the poor or servant classes. Testimony was uniform that the patients had not been away from home within six weeks preceding the onset, nor were their occupations, in most instances, such as to expose them to unusual contacts. It was not a question of a single urban center, for beside Montgomery and Mobile, cases were discovered in two small towns in south Alabama—Andalusia and Headland. The disease prevailed during summer, fall and early winter, and instead of increasing, disappeared as the cold weather advanced.

The clinical findings were not altogether typical of typhus. The disease was mild. Nervous symptoms were not in evidence. The eruption was polymorphous—macular or mapulo-papular rather than petechial—and in no case showed a distinctly hemorrhagic character. In case 12 the rash was evanescent; in case 13, rash was not noted by the attending physician or patient. The fever ended by lysis rather than by crisis except in 2 instances. The white blood cell count showed leucopenia in 3, a normal count in 2, of the 5 cases in which it was ascertained.

Therefore, in spite of the positive Weil-Felix reaction, it seemed possible that these cases might belong to some disease entity other than typhus. Consideration was given particularly to the possibility of their belonging to the typhoid group, with an unusual development of the skin manifestations, to Rocky Mountain spotted fever, to relapsing fever, or to dengue.

Typhoid seemed to be ruled out by the laboratory observations. In no instance did the patient's blood show any agglutinating power for the typhoid group of organisms. Rocky Mountain spotted fever was rejected as a possibility because of the lack of any epidemiological evidence to support tick transmission and the atypical clinical course. The absence of recurrence of fever and the leucocyte count spoke against the possibility of relapsing fever. This narrowed the consideration to dengue and some unknown or unrecognized disease—should they prove not to be typhus.

Dengue is endemic in the southern United States. During the period of these observations the country was suffering from one of the epidemic outbursts of this disease which occur every few years. Montgomery had a large number of cases of dengue occurring in the same sections of the city and at the same time as the cases reported above. All except cases 12 and 13 occurred while the insect vector (*Culex quinquefasciatus?* or *Aëdes aegypti?*) was active. These last two cases occurred in February just after the coldest weather of the year had been experienced and mosquitoes were in abeyance. No history of mosquito pest could be obtained from these patients.

On the basis of symptomatology, it will be found that this series of cases could be classed as dengue. The sudden onset, the rash developing on the third to the fifth day, the polymorphous character of the eruption, the suffusion of the conjunctivae, the characteristic complaint of headache, backache and pains in the extremities, nausea, uncomplicated course with uniform recovery, and finally the leucopenia—all of these are found in dengue, and "dengue-like" fevers which have been variously described under the terms "ten-day" "twelve-day" and "fourteen-day" fevers. However in the small series of cases of dengue lasting three to seven days in which the sera were tested for the Weil-Felix reaction, the results were negative.

This fact and the uniformly long duration of all the cases render the diagnosis of dengue improbable. In view of the facts so far adduced it would seem to be a reasonable hypothesis to place this series of cases occurring in Alabama in a single clinical syndrome giving a positive Weil-Felix reaction. On clinical and serological grounds, mild typhus or Brill's Disease seems to be the most likely possibility. On the other hand, if this diagnosis be correct, it is difficult to explain the mode of spread and the distribution of these cases.[4]

CONCLUSION

Eleven cases of fever of about two weeks duration, with eruption and developing a positive Weil-Felix reaction during the course of the illness, have been described. Evidence has been presented to indicate doubt as to the proper classification of these cases; although Brill's disease seems to be the most likely possibility.

CONCLUSIÓN

Se describen once casos de fiebre de cerca de dos semanas de duración, con erupción y una progresivamente positiva reacción de Weil-Felix. La evidencia indica lo dudoso de la clasificación propia de estos casos.

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[4] Since writing this paper, more cases have come to the attention of the authors and the study is being continued.

THE COMPLEMENT FIXATION TEST AND OTHER FINDINGS IN MALARIA[1]

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The following résumé of findings in cases of malaria was prompted by the recently reported employment of *Plasmodium vivax* in the treatment of general paresis. Wagner-Jauregg (1) noting numerous reports of improvement in paresis resulting from infectious febrile conditions, first proposed *Plasmodium vivax* inoculation for the treatment of paresis in 1887 and later used Koch's tuberculin in doses at first small but in later cases quite large. Typhoid vaccine in large doses was also employed and both these gave varying degrees of improvement. In 1917 he inoculated several paretics with *Plasmodium vivax* and obtained a degree of improvement that warranted the continuation of the method. A soldier infected with benign tertian malaria and untreated with quinine, served as donor, and 1 to 4 cc. of his blood was injected subcutaneously into a group of paretics. They were permitted to have several chills, served as donors for other cases of paresis, and were then given quinine. At the time of his report the strain of *Plasmodium vivax* had been transferred 37 times through 200 paretics. Successful inoculation was also secured by rubbing a few drops of the infected blood upon scarified areas of the skin. All the inoculated cases were readily cured of the malaria, the parasite being apparently very susceptible to quinine.

Recently the treatment of paresis by malaria inoculation has been undertaken at St. Elizabeth's Hospital. For this work the endeavor was made at the Naval Hospital, Washington, to secure a patient with benign tertian malaria, without a history of syphilis, willing to give blood, and with a negative Noguchi reaction. The laboratory work on the prospective donors was done in the laboratories of the Naval Medical School, and the fulfillment of the latter requirement showed some interesting reactions and suggested a review of the laboratory records in cases of malaria.

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[1] Read by abstract at the nineteenth annual meeting of the American Society of Tropical Medicine, San Francisco, Calif., June 25, 26, 1923.

Opinions differ widely as to the effects of malaria on the Wassermann reaction. In the numerous reports on the subject the authors rarely give an adequate description of the technique employed in their work so that it seems better to refer to the test as a complement fixation test rather than a Wassermann test as very few employed the Wassermann technique. Reports by de Jong (2) (3), Thompson (4), St. John (5), and Johnson (6), all stress the importance of basing conclusions only on tests performed according to well established technique. St. John used cholesterinized antigens, with modified Noguchi technique. Johnson employed the standard Wassermann method on all sera, with parallel tests in other laboratories by at least one of three other methods in each case, and obtained complete agreement in only 57 per cent of the tests.

The occurrence of a positive complement fixation test for syphilis, in cases of malaria has been reported by Craig (7), Sutherland (8), de Jong, St. John and Hehewerth (9). Craig in a review of 2643 Wassermann reactions considered only 5 due to malaria. Wassermann and Lange (10) feel that a positive reaction in anyone who has come from a malaria country is not acceptable evidence of syphilis unless the test remains positive after quinine therapy. They refer to the work of Meyer and Bonfiglio in Italy who obtained positive tests in 80 per cent of their cases of malaria. Schoo (11) obtained 22 positive reactions in 28 cases of malaria, Zschuki (12) reported 14 of 27 positive, de Haan (13) 63 of 153 positive and felt that syphilis was excluded in 29 of the positive cases. Meyerstein (14) does not give the number of cases tested but reports from 70 to 80 per cent positive, with the strongest reaction in blood taken during the febrile stage, a positive reaction ten days later being very rare and all reactions negative after quinine therapy.

Hehewerth tested heated and unheated serum from Europeans and from the natives of Java. With heated serum he obtained no positive reactions on 3 Europeans (3 of them having treated syphilis) while all 3 were positive with unheated serum. Unheated serum among the natives gave many more positive tests than heated and the positive reactions persisted for some time, many over six months, but the inhibition of hemolysis usually became weaker. They conclude that in a malarious country a positive Wassermann cannot be considered evidence of syphilis in the absence of positive clinical evidence of syphilis.

Opposed to these reports of positive reactions due to malaria there is a smaller number of reports, but included in this group are some noteworthy series. Thompson (4), using the Wassermann technique in a well established laboratory tested the serum of 130 individuals, with negative tests in 122. Fifty-two of the patients had parasites in the blood at the time of the test, and blood was taken at varying times for different groups. The first group during the stage of chill, and successive groups ranging as high as several weeks after the last observed chill. One such series was made on patients receiving quinine and another on patients receiving no quinine. The serum from 70 patients not showing parasites at the time of the test but with a history of malaria was tested with 70 negative results. Eight cases of malaria giving a positive reaction were referred to C. H. Mills and he pronounced all of them syphilitic after thorough examination. Thompson draws conclusions that seem entirely justified by his results, namely: 1. The Wassermann reaction conducted according to a recognized standard method does not give a positive reaction in malaria at any stage of the disease. 2. If a positive Wassermann reaction is obtained in a case of malaria, it is either due to undiagnosed syphilis or to a faulty technique.

Johnson (6) attributes the varied opinion as to the occurrence of positive tests in malaria as due principally to the varieties of complement fixation tests used. He concludes: 1. That the blood in active malaria does not give a positive Wassermann. 2. Syphilis should not however be diagnosed on the occurrence of a positive reaction on blood taken during the febrile stage of malaria. 3. If the test is again positive on blood taken later it furnishes the usual accepted evidence of syphilis.

Thompson, de Jong and Johnson all emphasize the point that there should be no clinical confusion from the occurrence of a positive test on blood taken during the febrile stage of malaria, as that is not the time to elect for the serum diagnosis of syphilis. Clinically the test as a diagnostic measure would not be performed at that time and hence there is no lessening of its value as an indication of syphilis in malaria patients.

In reviewing the laboratory records of the Naval Medical School it was found that since January, 1917, there were recorded 68 cases in which the presence of malarial parasites was demonstrated in the blood. Of these, 57 were benign tertian and 11 estivo-autumnal. The complement fixation test employed here is the modification of Noguchi, using an acetone insoluble antigen and a cholesterinized antigen with heated serum. The technique is well established and on several occasions has given results practically identical with tests conducted in other laboratories on samples of the same serum separated for comparative tests. In the 68 malaria patients a complement fixation test was done on the serum of 34. All of these were negative with both antigens except 4. Two of the 4 cases had a definite history of syphilis, and a record of treatment with arsenicals and mercury. When their blood was taken, they were in the febrile stage of a paroxysm with very numerous benign tertian parasites in the peripheral circulation. The syphilitic patients showed only partial inhibition (acetone insoluble antigen 1+ and 2+), cholesterinized antigen 2+ and 3+ respectively) comparable to the readings in the same series of tests, on malaria-free patients under syphilitic treatment. Both patients after receiving quinine by mouth for from five to seven days, gave a negative complement fixation test and showed no parasites in the peripheral circulation on prolonged and repeated search. More of the patients in the group with negative Noguchi reactions probably had treated syphilis, but as their hospital records are not available, an exact statement can only be made in regard to 3.

The third patient had blood taken twenty-four and forty-eight hours after a chill, the second specimen during the cold stage of the next paroxysm. This serum was held forty-eight and twenty-four hours and each specimen gave a partial inhibition (1+ acetone insoluble, 2+ cholesterinized antigen). The same serum was kept in a refrigerator for four days more and tests on both were negative, which does not confirm the opinion of Thompson that heated serum tends to become positive when kept. This patient after receiving quinine for five days gave a negative complement fixation test on fresh heated serum. He was at that time free from parasites. A careful inquiry into his history and careful physical examination by competent clinicians revealed no evidence of syphilis. Several later tests on this patient were negative. It is believed that this reaction was not due to faulty technique as it is extremely unlikely that two mistakes would be made in one run of tests, nor if made, that they would occur in tubes, in widely separated positions in the rack. The same series of tests carried clear cut positives and negatives in addition to the controls, and tests on a group of syphilitic patients at varying stages of intensive hospital treatment and showing the degree of inhibition of hemolysis expected at their respective treatment stages.

Another patient, with a history of initial malaria infection in 1921, has had numerous recurrences, with a record of 4 negative Noguchi reactions at various Naval laboratories. In all these laboratories the complement fixation technique is the same as that employed in this laboratory and their antigen and amboceptor were produced here. One of the negative tests was performed here on blood taken a few hours after a chill in a previous attack. On blood taken within thirty minutes of the onset of a chill, showing very numerous parasites of benign tertian malaria, a 3+ reaction with acetone insoluble antigen and complete inhibition (4+) with cholesterinized antigen, was the result. Blood taken twenty-two hours after the paroxysm gave a 2+ and 3+ reading. Both serums were kept in the refrigerator for four days and tested again with negative reactions. This patient also gave no evidence of syphilis on careful examination. Serum taken after five days of quinine gave a 1+ and 3+ reaction.

So excluding two known syphilitics with malaria, there have been only 2 positive complement fixation tests in 32 patients. As most of the patients had more than one test the actual number of Noguchi tests were approximately 55.

One interesting clinical observation was that at least 2 of the patients having malaria, had recently completed a course of arsenical treatment for syphilis, without eradication of the *Plasmodium vivax*. This was evident from the recurrence of symptoms of malaria at a season and location precluding new infection.

In reviewing the laboratory records, there were recorded 38 complete blood counts made at the time of finding the malaria parasites. A few of the counts showed very marked increase in the percentage of large mononuclear cells, but the average of the counts was as follows:

Erythrocytes	4,759,000
Leukocytes	7,670
Polymorphonuclears	60.8 per cent
Lymphocytes	28.4 per cent
Large mononuclears	9.2 per cent
Eosinophiles	1.2 per cent
Basophiles	0.3 per cent

There is possibly a slight increase in the percentage of large mononuclears but the count tends to confirm the recent report of Swan (15) in which he considered a large mononuclear increase as not constant during the active stage of malaria.

Our records of complement fixation tests seemed to confirm the opinion that a test conducted according to a standardized technique, on the serum of malaria patients will be negative in the absence of syphilis. However the last two patients, appearing while this paper was in preparation, and showing at least partial inhibition of hemolysis in more than one portion of serum necessitates the conclusion that occasionally serum taken during active schizogony may yield a positive Noguchi test, but that it is very uncommon. In view of the two positives in cases of treated syphilis, both with negative Noguchi's prior to the onset of malaria, we feel that we have additional evidence that a positive Noguchi in malaria is due to syphilis in the great majority of cases. Of course the impropriety of doing a Noguchi test on a patient during his active malaria as a diagnostic test should rule out any improper assumption of syphilis as a result of the very rare positive test.

CONCLUSIONS

1. Large monunclears are slightly if at all increased in the active stage of malaria.
2. Careful technique will eliminate a number of the positive complement fixation tests in malaria.
3. Rarely there is a partial inhibition of hemolysis in serum taken during active schizogony.
4. Because of this rare positive, no attempt to diagnose syphilis by complement fixation test in a malaria patient should be made till after the peripheral circulation has been freed of demonstrable parasites by quinine.
5. Patients with treated syphilis and a negative Noguchi may show a weakly positive reaction if their serum is taken during a malarial paroxysm, but the majority will not.

CONCLUSIONES

1. Durante los períodos activos de la malaria, los mononucleares grandes se aumentan muy poco, si acaso.
2. Una técnica cuidadosa elimina un cierto número de las reacciones positivas por la fijación del complemento en la malaria.
3. Durante la esquizogonia activa muy rara vez se nota la inhibición de hemólisis en el suero.
4. Debido a esto, no se debe tratar de hacer un diagnóstico de sífilis, en pacientes maláricos, por la fijación del complemento mientras tanto no se haya librado a la circulación periférica de parásitos por medio de la quinina.
5. Los sifilíticos ya tratados, y con reacción Noguchi negativa, puede que aun reaccionen débilmente positivos si el suero se obtuvo durante un paroxismo malárico. La mayoría de los casos, sinembargo, no reaccionan así.

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STUDIES OF MALARIA IN PANAMA

V. PERNICIOUS MALARIA[1]

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Pernicious malaria has been subdivided by various authors into different types on the basis of predominant symptoms, and this practice has led to a bewildering number of ill-defined groups such as the "algid, bilious, cardialgic, choleraic, comatose, delirious, dysenteric, eclamptic, hemiplegic, hemorrhagic and pneumonic" (1). As many as thirty different types have seen described.

Furthermore, there have been no definite criteria proposed for separating malarial attacks of moderate severity from "pernicious" attacks, and so there has been no unanimity of opinion as to what constitutes "pernicious" malaria other than that it is an illness of such severity "as to endanger the life or actually cause the death of the patient."

It seems reasonable to believe that in Panama, with the enormous material observed under hospital conditions and with the intense infections that occurred, we should have seen malarial infections run the gamut of malarial phenomena. After six years of experience there, we concluded that in addition to the usual subdivisions of pernicious malaria which can be accounted for by prominent symptoms, there are others which can only be explained by the theory that certain complications have been attributed mistakenly to malaria. For example, in a previous study (2) of the relation of malaria to other diseases, evidence was presented indicating that the "dysenteric" type of pernicious malaria did not occur in Panama, and that not only did both amebic and acute dysentery tend epecially to determine an exacerbation of latent malaria, but that typhoid fever and pneumonia, also, did likewise, although to a lesser degree. Therefore, in a relatively high percentage of these diseases, malarial parasites could be demonstrated in the blood.

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As our experience increased, it gradually became apparent, through the correlation of clinical observations and post mortem studies, that the "pernicious" attacks fell into three fairly well defined pathological groups which could be recognized clinically by certain criteria, and, furthermore, that the grouping was of practical value in reference to the prognosis and the indications for or against quinin treatment.

MATERIAL

The material studied included 239 attacks of erythrolytic hemoglobinuria (blackwater fever), the treatment (3) and etiology (4) of which have been discussed in previous papers, and 92 other malarial infections which were considered "pernicious" and which occurred in Colon Hospital between October, 1907, and May, 1911. The hemoglobinuric group was fairly clear from the first (although it was found later to overlap the other groups), and the 239 attacks cover a longer period of time and include cases that occurred in both Ancon and Colon Hospital.

The subdivisions and criteria to be proposed and discussed were not correlated during the period of the first 60 of the 92 cases of "pernicious" malaria, and so only the last 32 are analyzed in detail in this study. There were 41 deaths among the 92 patients, a mortality of 44.5 per cent.

SUBDIVISIONS AND CRITERIA

The subdivisions which are proposed in this paper constitute rather a rearrangement and simplification than anything new. They are based upon predominating pathological phenomena instead of predominating symptoms. The three groups are as follows:

1. Hyperinfection.
2. Cerebral thrombosis.
3. Erythrolytic hemoglobinuria.

The groups, although overlapping, can be recognized fairly definitely by a study of three clinical phenomena, which are proposed as differential criteria.

1. Degree of infection—6 per cent or more of the red blood corpuscles infected.
2. Cerebral symptoms—coma or stupor.
3. Hemoglobinuria—trace to "blackwater."

TABLE 1

General statistics

GROUP	NUMBER OF CASES	RED BLOOD CELLS INFECTED	COMA OR STUPOR	HEMOGLOBINURIA	MORTALITY
		per cent			*per cent*
1. Hyperinfection	22	6–66.2	16	20	32
2. Cerebral thrombosis	10	1– 4	10	6	70
3. Erthrolytic hemoglobinuria	239	0– 6	0	239	16.7

DISCUSSION OF THE INDIVIDUAL GROUPS

Group 1, hyperinfection. The predominating feature of this group is the intense infection, determined by a differential count of the infected and uninfected red blood corpuscles. This count should be made at a time when the plasmodia are most numerous in the peripheral circulation, that is, a few hours after the onset of the paroxysm when the young forms of the sporulating group have been liberated, and before they are old or large enough to be "strained out" of the circulation by the capillaries. For example, the blood of one patient when first counted, during the morning hours, showed about a 1 per cent infection. A paroxysm began about noon, and a count made late in the afternoon showed more than a 6 per cent infection.

After long experience, including many determinations of the percentage of infected corpuscles and their relation to the appearance of hemoglobin in the urine, we accepted as a standard of hyperinfection an infection of 6 per cent or greater of the red corpuscles. In a previous paper (3), we reported having found hemoglobin in the urine of eight successive cases of pernicious malaria (not of the "blackwater fever" type), and that the degree of hemoglobinuria varied from a trace, demonstrable by the guaiac and turpentine test, to the intensity of "blackwater." Subsequent observations showed that almost invariably when the intensity of infection exceeded 6 per cent, hemoglobin appeared in the urine, there being only two exceptions in the 22 cases reported here. One of these 2 patients had 17 per cent of his corpuscles infected. He died before the sporulation of this large group of parasites occurred. Otherwise, it is fairly certain that in his urine also hemoglobin would have been demonstrable.

A 6 per cent infection of the red blood corpuscles appears, therefore, to mark the threshold of hemoglobinuria in malarial attacks not of the "erythrolytic" type.

Since, moreover, patients with a 6 per cent infection always seemed extremely ill and on the borderline of danger, that degree of infection was chosen as affording an objective criterion of pernicious malaria of the hyperinfection group.

Table 2 shows that 7 of the 11 patients who had more than 10 per cent of their corpuscles infected died, while none of those died who had an infection of 10 per cent or less. All of those who developed marked coma died, but 3 of the semi-comatose recovered, 1 of whom had 29 per cent of his corpuscles infected. The intensity of hemoglobinuria bore no direct relation to the intensity of infection or to the prognosis. Four of the five patients with hemoglobinuria of "blackwater" intensity recovered. It seems possible, even, that extensive erythrolysis may be accompanied by a destruction of the parasites, as seems to be the case in erythrolytic hemoglobinuria, and may be a favorable complication. In infections belonging to this group, therefore, cerebral thrombosis, manifested by coma, is the danger to be feared, and every effort should be made to prevent such a catastrophe. When an infection with young parasites of 10 per cent or greater is discovered, the attempt should be made by giving quinin intravenously, to kill the parasites in the intracorpuscular stage, and thus to prevent their growth to a stage when they are likely to cause thrombosis of the capillaries of the brain. According to Bass and Johns (5) malarial parasites are firmer in consistency than red blood corpuscles and their rigid bodies interfere with the normal elasticity of the cells, causing the latter to stick in the narrow capillaries and block the blood channels of the brain.

Quinin in ordinary doses probably does not affect markedly the intracorpuscular parasites. However, there is clinical evidence not only that the growth of the parasites may be inhibited and the parasites destroyed but also that many of the infected corpuscles may be saved (3). In these dangerous cases, 10 to 20 grains of quinin in the form of *bimuriate with urea* or the *dihydrochloride* may be given intravenously every four to eight hours until the patient is out of danger. We injected slowly the quinin dissolved in 15 to 20 cc. of normal salt solution, but we believe

TABLE 2

Statistics of group 1—hyperinfection

CASE	PERCENTAGE OF RED BLOOD CELLS INFECTED	COMA (++), STUPOR (+)	HEMOGLOBINURIA (TRACE + TO BLACK-WATER +++)	RESULT
1	66.2	++	+	Death
2	43	++	++	Death
3	29	+	+	Recovery
4	23.5	++	++	Death
5	15–20*	+	+++	Recovery
6	17.6	+	++	Recovery
7	17	++	0	Death
8	15.6	0	+++	Recovery
9	15.4	++	++	Death
10	12.6	++	+++	Death
11	11.4	++	++	Death
12	10	0	+++	Recovery
13	9	0	+++	Recovery
14	9	+	++	Recovery
15	8–10*	0	0	Recovery
16	5–10*	+	++	Recovery
17	5–10*	+	+	Recovery
18	5–10*	+	+++	Recovery
19	5–10*	+	+	Recovery
20	5–10*	0	+	Recovery
21	5–10*	+	++	Recovery
22	6.2	0	+	Recovery

* Estimated.

that it should be diluted more than we diluted it, and we suggest about 100 cc. of salt solution and that at least ten minutes be allowed for its injection.

Since the development in recent years of blood grouping and an adequate technique of blood transfusion, it is probable that many of these patients with hyperinfection could be saved by repeated bleedings and large transfusions. If by this procedure the infection should be reduced to 10 per cent or less, quinin by intravenous injection should then be able to save most of the patients.

TABLE 3

Statistics of group 2—cerebral thrombosis

CASE	PERCENTAGE OF RED BLOOD CELLS INFECTED	COMA (++), STUPOR (+)	HEMOGLOBINURIA (TRACE + TO BLACK-WATER +++)	RESULT
1	3–4*	++	+	Death
2	3.2	+	+++	Recovery
3	3	++	+	Death
4	2–3*	++	+	Death
5	2.3	++	0	Death
6	2	++	0	Recovery
7	1–2.5*	++	0	Recovery
8	1–2.5*	++	+	Death
9	1–2.5*	++	0	Death
10	1–2.5*	++	+++	Death

* Estimated.

Group 2, cerebral thrombosis. This group is characterized by thrombosis of the cerebral capillaries, manifested clinically by coma or stupor, and a relatively small number of malarial parasites in the peripheral circulation. Hemoglobinuria may or may not be present, and may vary from a trace to blackwater intensity.

There were 10 patients in group 2, and 7 of them died, a mortality of 70 per cent. All except 1 were deeply comatose. In the urine of 6, hemoglobinuria could be demonstrated. In 2 instances (cases 2 and 10), the hemoglobinuria was of blackwater intensity, and there was some doubt as to whether these 2 patients belonged to the cerebral thrombosis group or the erythrolytic hemoglobinuric group. Indeed, they seemed to be clinical links between the two groups. One of them (case 10) died and the brain capillaries were found to be extensively thrombosed and contained many parasites.

In the treatment of group 2, we used intravenous injections of quinine as described under group 1, but without marked success. A method of treatment that has more recently suggested itself to us is to drain the cerebrospinal fluid and transfuse the patient. The first procedure might very well dilate the capillaries of the brain and the second might, by increasing the blood volume, help to dislodge infected corpuscles caught in the capillaries.

Group 3, erythrolytic hemoglobinuria. This group is characterized by intense erythrolysis (manifested clinically by hemoglobinuria of "blackwater" intensity), by the absence of marked cerebral symptoms, and by only a few or no malarial parasites demonstrable in the peripheral circulation.

The 239 attacks of erythrolytic hemoglobinuria included in this paper have been reported and discussed in detail in two former papers (3, 4). In the latter paper we presented some evidence in support of the view that malaria is the essential etiologic factor. In 3 instances of very heavy malarial infections, we laked the blood rapidly, centrifugalized out at high speed the stromata with parasites attached or included, poured off the supernatant fluid, and extracted the sediment with sterile water, and added an equal quantity of 1.8 per cent salt solution. Tests showed that this extract contained an erythrolysin of considerable strength. The second and third extracts were studied in some detail and briefly reported (4). To quote:

> Three parts of the extracts hemolyzed completely one part of a 5 per cent suspension of erythrocytes in from twenty minutes to one hour. The hemolysin was thermolabile, its action was inhibited by one part of serum from normal persons, from 1 patient with pernicious malaria (the patient from whom one extract was obtained), and from 2 patients with erythrolytic hemoglobinuria. The antihemolysin in these serums was thermostabile. The serums were treated with quinin and tested for antihemolytic properties. The normal serums were unimpaired, but the serums from erythrolytic hemoglobinuric patients seemed to be weakened by the same quantity of quinin and in one instance hemolysis was complete in one hour. The experiments need

confirmation and we hope to be able to report more exact quantitative work. They seem to reconcile the malarial and quinin views of erythrolytic hemoglobinuria and to connect it definitely with malaria. The therapeutic measure of transfusion with normal blood is obviously suggested.

Owing to a change of residence to a non-malarial district, the above investigation has not been pursued. However, since Bass and Johns (5) have succeeded in cultivating malarial organisms it should be feasible to obtain and study their erythrolysins, if such exist, and probably the question of the relation of malaria to blackwater fever could be definitely settled.

Our observations indicate that the estivo-autumnal malarial parasite does generate an erythrolysin which is probably an enzyme, that its action is inhibited by anti-substances in sera, and that these anti-substances, in turn, may be influenced or partially inhibited by quinine and probably by other agencies such as exhaustion, exposure and prolonged malarial poisoning.

Causes of death. Deaths in groups 1 and 2 are due in the great majority of cases to thrombosis of the capillaries of the central nervous system—34 of 41 deaths in the series of 92 cases. A few deaths, however, are due to complications, or, in group 1, to the overwhelming toxemia of intense infections. Among the complications contributing to death in the series of 92 cases, were lobar pneumonia, 1 case; ruptured spleen, 1 case; miliary abscesses of the spleen, indicating a terminating infection, 1 case; another terminating infection with an organism apparently belonging to the typhoid-colon group, 1 case. Two other patients died after the temperature became normal and after the malarial infections had been eradicated. At the postmortem examination, there were no gross lesions to account for death, no thrombosis of the cerebral capillaries, and no demonstrable malarial parasites. Microscopically, the kidneys showed a parenchymatous nephritis. However, we did not feel that the nephritis was the cause of death, but rather that it was a part of the sequelae of an overwhelming toxemia from the effects of which the cells of the body could not recover. Case 2, of group 1 (table 2) supports this view. The patient had 43 per cent of the corpuscles in the peripheral blood infected, each with 1 to 6 parasites. In spite of such an infection (the second largest that we saw) he lived seven days and died after his temperature had been normal for three days. The day before death he voided 840 cc. of urine, and on the day of death his urine contained no albumin. At postmortem, no parasites were found in the brain or other organs, there was no thrombosis of the brain capillaries, and there were no gross lesions other than pigmentation of the viscera and enlargement of the spleen. It seemed that the patient had been unable to recover from the effects of the enormous infection.

Death in group 3, erythrolytic hemoglobinuria, is usually due to an intense nephritis. The gravity of the prognosis increases with a decreased urinary output, and the majority of patients who die have suppression of the urine. A smaller number die from the intense destruction of erythrocytes and the sudden anemia. The mortality of the 239 attacks of group 3 was 16.7 per cent. We feel that this percentage could be tremendously decreased by withholding quinin and giving large transfusions of normal blood. The transfusions not only would combat the sudden anemia, which may be considered equivalent to that following a large hemorrhage, but, if our observations on malarial hemolysin and antihemolysin are correct, they would furnish the antihemolysin impaired in the patients blood and tend to prevent further erythrolysis.

Parasites of pernicious malaria. The estivo-autumnal malarial parasite was present in 91 of the 92 cases of pernicious malaria of groups 1 and 2, and it was the only one found in the cases of group 3. In 1 case of group 2, a double tertian infection of moderate intensity was present. The patient, a negro boy, aged seventeen, was comatose on admission to the hospital and lived only fifteen hours, although treated intensively with quinin. The postmortem examination showed that the cerebral capillaries were thrombosed. No parasites could be found in smears from the brain, bone marrow or spleen, although a few tertian-like forms were found in the blood smear from a meningeal vein. A culture from the splenic pulp was negative. A trace of hemoglobin was present in the urine. Altogether the case must be considered one of fatal pernicious tertian malaria. We have observed coma in one other tertian infection not included in this series. The patient was a young negro man, the infection was enormous, and coma developed during the paroxysm. The patient recovered rapidly when large doses of quinin were given by intramuscular injection.

SUMMARY

1. The material studied included 331 attacks of pernicious malaria.

2. Three pathological subdivisions of pernicious malaria are suggested to replace the numerous old subdivisions based on predominant symptoms or complications erroneously attributed to malaria. The subdivisions or groups are as follows: (1) hyperinfection; (2) cerebral thrombosis, and (3) erythrolytic hemoglobinuria.

3. Three clinical criteria are proposed for recognizing the pathological groups: (1) degree of infection—6 per cent or more of the red blood corpuscles infected; (2) cerebral symptoms—coma or stupor, and (3) hemoglobinuria—trace to "blackwater."

4. A statistical study of the three groups is given, and treatment discussed.

5. The causes of death and the parasites of pernicious malaria are discussed.

SUMARIO

1. El material estudiado incluye 331 ataques de malaria perniciosa.

2. Se sugieren tres subdivisiones patológicas de la malaria perniciosa para reemplazar las numerosas del pasado que se basan en síntomas predominantes o en complicaciones erróneamente atribuídas a la malaria. Las subdivisiones o grupos son los siguientes: (1) hiperinfección; (2) trombosis cerebral; (3) hemoglobinuria eritrolítica.

3. Se proponen tres criterios clínicos para reconocer los grupos patológicos: (1) grado de infección (6 por ciento o mayor proporción de infección de los corpúsculos rojos); (2) Síntomas cere-

brales (coma o estupor); (3) Hemoglobinuria (desde un vestigio hasta "blackwater").

4. Se dá un estudio esta dístico de los tres grupos y se arguye la cuestión del tratamiento.

5. Se presenta una discusión acerca de los parásitos de la

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THE CULTIVATION OF A RICKETTSIA-LIKE MICRO-ORGANISM FROM TSUTSU-GAMUSHI DISEASE

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In the summer months, a severe infection known as "tsutsugamushi," or Japanese flood river fever, is prevalent in certain valleys on the west coast of Honshiu, the principal island of Japan. During the heavy summer rains in the mountains in the background of this region, the rivers of these valleys flood their low lying banks. After the water subsides, certain species of mites known locally as akamushi, or red bug, make their appearance in the recently flooded areas. The name "tsutsugamushi" signifies "dangerous bug." The bite of these mites ordinarily produces only local inflammation. In case the mites harbor the virus of the disease, a sharply defined ulcer several millimeters in diameter develops after a few days. The regional lymph glands become markedly inflamed; then about a week after the original bite, the temperature rises sharply. With the onset of fever a generalized eruption makes its appearance reaching its maximum intensity in a few days and fades before the temperature becomes normal. The febrile reaction persists in the average case for about two or three weeks, the temperature returning only very gradually to normal. The pulse rate is proportional to the temperature; a well developed leucopenia usually accompanies the febrile reaction. A variety of nervous symptoms not unlike those of typhoid fever appear in the severe cases. The mortality of the disease as it occurs in Japan is high, varying from 10 to 62 per cent (1). One attack confers a moderate degree of immunity. Nothing remarkable has been noted in the pathological examinations. Kawamura, Arima and

Hattori (2) described the primary lesion as a coagulation necrosis walled off by an exudate of fibrin and cells. The eruption in the skin shows the usual infiltration of leucocytes. The spleen and lymphatic glands are enlarged, the latter frequently showing small hemorrhages.

Microörganisms in considerable variety have been described as the cause of the disease but according to the consensus of opinion the etiologic agent is entirely unknown.

One is at once impressed by the similarity in the clinical picture between tsutsugamushi disease and typhus fever as well as spotted fever of the Rocky Mountains. The characteristics of the Japanese disease are sufficiently distinctive, however, to establish it as a distinct entity. This conclusion is amply supported by the extensive experimental work of many Japanese observers. Kitashima and Miyajima (1) infected Japanese monkeys (*Macacus fuscatus*) by injection with blood from a patient and also by exposing the monkeys to the bite of mites. The first injection of monkeys with patients' blood sometimes fails to produce an infection; once the disease is established in animals, the individual monkeys (*M. fuscatus*) react definitely with remarkable regularity. The disease is characterized in monkeys by a fever and leucopenia. No generalized eruption occurs but a local lesion develops in the monkeys bitten by mites. For some months (or even years) after the successful infection of a monkey, the re-injection of virulent blood produces no reaction. These authors also made a contribution which is extremely valuable in the study of the virus in the laboratory. They found that guinea-pigs although showing no febrile reaction nor leucopenia, nevertheless conserve the virus for a period of at least twelve to fourteen days. Moreover the virus may be subinoculated several times through a series of guinea-pigs without losing its virulence. A monkey when inoculated with blood from an injected guinea-pig develops a typical fever and leucopenia. Therefore it is possible to maintain a strain of the virus without depending entirely on monkeys. Furthermore this experiment is of value in differentiating this virus from closely related infections. Thus, in the guinea-pig, tsutsugamushi disease produces no symptoms; typhus fever produces a temperature reaction as well as characteristic lesions of the brain but the animals ordinarily survive; spotted fever produces a temperature reaction and the animals usually die. In the case of trench fever it is assumed that guinea-pigs do not even conserve the virus; however this point has not been thoroughly tested since there is no method of demonstrating this virus at present except by the inoculation of human beings.

In the summer of 1922, the writer had the opportunity of visiting Japan. Through the courtesy of Drs. S. Hata and S. Kusama of the Kitasato Institute, a visit was arranged to the principal endemic focus of tsutsugamushi disease in Niigata. In addition to the many courtesies which were extended by the Japanese officials, Dr. R. Kawamura very kindly provided me with a strain of tsutsugamushi virus. This had been secured two years previously by the injection of patients' blood into monkeys (*M. fuscatus*). Subsequently the virus had been propagated by subpassage in guinea-pigs and monkeys. Transfers from guinea-pig to guinea-pig were made at thirteen-day intervals using blood and splenic tissue, injecting the material subcutaneously. After the third passage in guinea-pigs the virus was always inoculated in a monkey to insure the preservation of its virulence. At the height of the febrile reaction in the monkey, blood was withdrawn and injected into guinea-pigs. A 1 per cent solution of sodium citrate in physiological saline containing 1 per cent of glucose was used for collecting the blood of the pigs and monkeys. Under these conditions Dr. Kawamura found that the virus maintained its virulence, unimpaired, giving sharp characteristic reactions (3).

I have followed this technique closely with one exception. It has been necessary to use the *M. rhesus* instead of the *M. fuscatus*. The Japanese observers have found that the latter species gives more characteristic reactions than any other of the macacus monkeys; nevertheless the reaction in the rhesus is fairly satisfactory. As a general rule, moderately sharp febrile reactions have developed accompanied by a drop in the leucocyte count giving a picture that is usually uncomplicated and reasonably easy to interpret. At the height of the reaction the monkeys appear somewhat ill though they do not lose their appetite. The transfers from the guinea-pigs have been made usually on the eleventh or twelfth day.

Pathology. Preliminary examinations were made for any evident pathological lesions. Guinea-pigs which were sacrificed for the passage of the virus frequently though not constantly showed induration of the tissues at the site of inoculation. The neighboring lymphatic glands and the spleen were often enlarged. Histologically the glands showed hyperplasia; the endothelial lining of the vessels was not swollen. No significant changes were found in the spleen. At this stage of the disease sections of the brain showed no lesions, in contrast to the changes which occur in typhus fever.

One interesting feature was noted in guinea-pigs, namely, the occurrence of hemorrhages, often distinctly separated from the site of inoculation. These were sometimes extensive and diffuse in the subcutaneous tissue. For example the blood and splenic tissue of an infected guinea-pig was injected under the skin in the right umbilical region of another pig; on sacrificing this animal twelve days later, hemorrhages were found in the right inguinal and femoral and also in the left inguinal regions. On several occasions, moderately large hemorrhages have been noted in the axilla. These hemorrhages also occurred after the injection of infective blood from monkeys. Thus far 65 guinea-pigs have been sacrificed at intervals of eleven or twelve days after inoculation for the passage of the virus; of this number twenty-four have shown hemorrhagic lesions more or less remote from the site of inoculation. Sometimes instead of diffuse subcutaneous distribution, numerous discrete punctate hemorrhages occurred in the muscles. One animal which at first glance appeared free from lesions showed these multiple punctate hemorrhages in the muscles of the abdominal wall but they were not evident until the fasciae or peritoneum was dissected away; in another instance similar hemorrhages were clearly visible from the peritoneal surface. In a single animal, hemorrhagic spots about 1 to 2 mm. in diameter, were grouped in lines along the course of the nerves on one side of the abdomen.

As a control for these observations, several series of guinea-pigs were injected subcutaneously in the right umbilical region with the blood and spleen of normal guinea-pigs and the blood of normal monkeys.

The blood was collected in citrate glucose solution in the same manner as for the transfer of the virus, the spleens of guinea-pigs being ground in a mortar with the citrated guinea-pig blood. In animals which were sacrificed after two to four days, the injected blood was definitely dark in color and there was an extravasation of fresh blood in the immediately adjacent tissue. The majority of the animals were sacrificed at the end of eleven or twelve days in order to compare the results with the injection of infective blood. In all, 13 guinea-pigs received an injection of normal guinea-pig blood and splenic tissue; at the end of eleven days, 6 were negative and in 7 there were small blood-stained areas at the site of injection. This was limited sharply to the injected area except in one instance in which the subcutaneous tissues over the right knee were slightly stained with blood. Four guinea-pigs received normal guinea-pig blood only without the addition of splenic tissue. These were entirely negative at the end of twelve days whereas animals injected with infective guinea-pig blood without splenic tissue sometimes showed extensive hemorrhages. Seven guinea-pigs were injected with normal monkey blood. At the end of twelve days, 3 were entirely negative; 4 showed small hemorrhages limited sharply to the site of inoculation.

To recapitulate, the injection of normal blood and splenic tissue was followed frequently by small hemorrhages but these were limited, except in one instance to the site of inoculation. Infective blood frequently produced diffuse hemorrhages at a considerable distance from the point of injection. These differences suggest that the virus of tsutsugamushi disease is capable of producing subcutaneous hemorrhages in the guinea-pig. The punctate hemorrhagic lesions which occasionally follow the injection of the virus were not observed after the injection of normal blood.

In the enlarged lymph glands of the guinea-pig, minute hemorrhages are not uncommon but extensive ones have not been encountered.

In the monkey in two instances, enlarged lymph glands draining the site of inoculation were excised and extensive hemorrhages were present. In one animal, an extensive subcutaneous hemorrhage was plainly visible through the skin.

It would seem therefore that these hemorrhagic lesions occurring in experimental animals are analogous to the hemorrhages observed in man in the lymph glands and perhaps to the petechial lesions found in the skin.

Recognition of tsutsugamushi in animals. Spotted fever runs a fatal course in guinea-pigs with a characteristic pathology. In typhus fever, the temperature reactions may be indefinite; contaminating organisms occasionally complicate the picture so that more or less confusion existed at one time. Wolbach and Todd (4) have however clearly and unmistakably defined the criteria essential for the recognition of typhus in animals, especial emphasis being placed on the lesions occurring in the brain. A valuable guide for the recognition of the virus of tsutsugamushi is found in the conservation of the virus in guinea-pigs without febrile reaction, the development of fever and leucopenia in monkeys injected with infective blood of guinea-pigs or man, and the immunity test in monkeys. To this, one should add the occurrence of hemorrhagic lesions; these lesions will require thorough histological study to elucidate the underlying pathological process.

In the histological examination of tissues from infected animals, attention was directed primarily to the search for Rickettsia-like microörganisms. Occasionally in the large mononuclear cells of the swollen and hemorrhagic lymph glands, suggestive bodies were seen in the cytoplasm. The exact interpretation of such comparatively structureless forms presents great difficulties. Even in typhus fever the demonstration of *Rickettsia prowazeki* in human tissues, requires great care. In any case, confirmation of the histological findings by artificial cultures is much to be desired.

Cultures. Anaerobic tubes of hydrocele agar were prepared with and without the addition of normal guinea-pig kidney tissue. These were inoculated with blood and splenic tissue from infected pigs but the medium remained sterile. Tubes of whole guinea-pig blood, with and without inactivation at 56°C. for fifteen minutes were inoculated but no growth was obtained. One set of tissue cultures was prepared according to Wolbach's technique for spotted fever (9). A piece of infective guinea-pig spleen was placed in guinea-pig plasma. The usual tissue growth occurred but no definite microörganisms were recognized.

At the time of sacrificing guinea-pigs for the transfer of the virus, cultures were made as a routine from the spleen. Usually one plate of "chocolate" blood agar was meared with the cut edge of a piece of spleen; occasionally plates were inoculated with blood, a piece of enlarged lymph gland or a bit of subcutaneous tissue showing hemorrhage. The preliminary cultures were made on chocolate plates; ordinary standard agar was used as a basis, to which defibrinated horse blood (25 to 30 per cent) was added and the mixture was then heated for fifteen minutes in the Arnold sterilizer. The cultures were incubated aerobically at 37°C. The usual contaminations were frequently found such as the cocci and the ubiquitous diphtheroids.

In one instance, after five days incubation, smears from an apparently sterile plate showed numerous Gram-negative slender rods; not infrequently small clumps were found arranged somewhat after the manner of diphtheroids. Very commonly small unstained areas like vacuoles occurred in these organisms. From time to time, long wavy chains of irregular contour were found. Using a well-aged Loeffler's methylene blue, these organisms appeared singularly like the Rickettsia of typhus fever both in their morphology and staining reactions. The isolated organisms appeared as blue rods with purple granules at each pole. With Giemsa's stain (Azur II eosin 3 grams, Azur II 0.8 gram glycerine and methyl alcohol each 250 cc.) the results varied somewhat according to the details of technique. With 1:10 dilution in water, of the stock solution, the organisms overstained so that little detail could be seen. The ordinary forms were a deep purple, the very pale forms seen with methylene blue preparations being a faint pink. With a 1:40 dilution of Giemsa stain the results approximated those obtained with methylene blue except that the color differentiation was less sharp.

Subinoculations were made using the same lot of chocolate agar but no growth was obtained. The original plate showing growth was stored in the ice-box (temperature 10°C.) and the subinoculations were continued, some of the plates being smeared with the pulp and juice from spleen of a normal guinea-pig. Finally, ten days after the first appearance of growth, a subinoculation on an ordinary chocolate plate showed a few minute colonies, after three days' incubation. The microörganisms of this subculture were identical in staining and morphology with the original growth. Subcultures were continued without difficulty though only a feeble growth resulted. Transfers were then made to Martin's agar chocolated in a similar manner, using some of the stock lot of Martin's medium which was prepared in this laboratory for work on measles (5). On Martin's chocolate agar an extremely luxuriant yellowish brown growth developed after 48° incubation. In the subsequent work Martin's medium was substituted for the standard agar in the preparation of the chocolate plates.

On inoculation of this culture subcutaneously in guinea-pigs, hemorrhages were produced identical in appearance with those resulting from the inoculation of the virus. It was, however, extremely difficult to recover this organism from the injected guinea-pigs using either Martin's or ordinary chocolate plates inoculated with tissue showing hemorrhages, with lymph glands, spleen, or blood.

During the course of the transfer of the virus, this Rickettsia-like organism has been recovered in pure culture 4 times. These strains have been obtained at fairly wide intervals, namely, (1) from the spleen of a guinea-pig in the sixth passage of the virus in this laboratory; from the spleens of guinea-pigs (2) in the eighteenth and again (3) in the nineteenth passages and also (4) from the heart blood of a monkey in the twenty-first passage. The organism has also been definitely recognized in two other cultures from material of the ninth and fifteenth passages but it was contaminated with rapidly growing bacteria; attempts at isolation in pure culture were unsuccessful.

Subcultures. Thus far the primary isolation of this organism has been obtained only on chocolate agar. In ten instances, tubes of egg yolk medium (6) have been inoculated but remained sterile or were overgrown by contaminating diphtheroids. On subculture however, the stock strains grew luxuriantly on egg medium with the production of a yellowish brown pigment. At least a moderately good growth has been obtained on other nutrient rich media, namely, hydrocele agar, whole guinea pig blood and NNN medium as prepared with rabbit blood for the cultivation of Leishmania. Growth is usually abundant after forty-eight hours at 37°C. With 1 strain which has been under artificial cultivation for seven months, luxuriant colonies frequently develop in twenty-four hours. On a thoroughly moist plate of Martin's chocolate, the individual colonies are perfectly round with smooth edges, and measure 2 to 3 cm. in diameter; the yellowish brown pigmentation does not always appear uniformly distributed throughout the colony.

At room temperature, some slow growth takes place, as might be expected if this organism is adapted to an arthropod vector. On simpler media, the behavior depends in part on the length of time that the organism has been under artificial cultivation. For example, a freshly isolated strain failed to grow when inoculated (i.e., first subinoculation) on meat infusion agar (pH 7.6) that proved suitable for the growth of virulent pneumococcus. Neither did any growth take place on glucose (1 per cent) or glycerine (5 per cent) agar prepared from the same stock of medium. However, after three subinoculations on Martin's chocolate agar, moderate growth developed with but very little pigment production on plain agar; on glucose agar the usual yellowish brown pigment was formed. These simpler media were not adequate for the continuous propagation of these cultures. Subinoculations of 3 strains of this organism were made at intervals of two to three days. All of these strains had been maintained on Martin's chocolate agar. One had been under artificial cultivation for six months; the second had been subinoculated 8 times and the third 9 times. On meat infusion broth (pH 7.6), a heavy inoculation gave rise to a distinct clouding of the medium after two days; subinoculations of an ordinary loop full of this growth to fresh broth produced no visible turbidity. After 6 transfers on plain agar, growth either failed entirely or was insignificant in amount. On the fourth subinoculation on glycerine agar, no growth occurred with the first two cultures and almost none with the third. Glucose agar proved a much better medium for maintaining these cultures; pigment production was maintained well for the first 5 or 6 transfers and a fairly good growth was obtained even after 12 successive transplants.

On milk, the cultures grew slowly. After one week, coagulation took place but without acidification using brom cresol purple as indicator. On Hiss-serum water (1 part of horse serum and 3 parts of water) with the same indicator, glucose, maltose and dextrin were fermented with formation of acid and no gas; lactose and saccharose remained unchanged. The details of the fermentation reactions have not been completed.

Only a feeble growth is obtained in the absence of a liberal supply of air. On glucose hydrocele agar a heavy pigmented growth appeared on the surface; in stabs a faint line of growth was visible for a distance of 1 to 2 cm.

Morphology. The most striking feature in the morphology of this organism is its variability. The essential features can be seen at a glance in the illustrations (plate 1). When growing freely on nutrient rich media such as Martin's chocolate or egg medium, a fairly uniform size is maintained, the majority of the organisms appearing as slender rods with an average length of 1.3μ and a breadth estimated at 0.3μ (plate 1, fig. 1). Under other circumstances, for example in cultures five or six days old on Hiss-serum water, the size may diminish almost to the minimum which can be resolved by the microscope (plate 1, fig. 3). On the other hand, long wavy chains of irregular outline beaded with granules frequently make their appearance, in cultures which are growing actively. The presence of granules is not interpreted in any sense as an indication of degeneration. Occasionally relatively large round or oval granules (1 to 2μ) are found occurring either in the chains or lying free. Some of these oval forms stain more deeply at the ploes (plate 1, fig. 2) suggesting somewhat the ''olive-shaped'' bodies figured by Arkwright and Bacot. In extremely young cultures (fifteen hours old), numerous forms are seen which take almost no stain with Löffler's methylene blue and contain no granules. These are illustrated in plate 1, fig. 4. The evidence available at present indicates that they are young forms of this organism, a faint line or dot is visible in the center, the surrounding halo giving the appearance of a capsule. However, these bodies stained uniformly with Giemsa and no definite capsules could be demonstrated by the method of Huntoon or Hiss.

Motility. The observation at room temperature of twenty-four and forty-hours cultures from egg medium has not shown any indication of motility in the individual organisms nor in the filamentous forms.

Resistance. The thermal death point was found to correspond to that for the ordinary delicate organisms. Young cultures (forty-eight hours old) suspended in salt solution, were exposed to a temperature of 50°C. for fifteen minutes; only a very few colonies developed on the plates inoculated from this suspension after heating but the control furnished abundant growth. After ten minutes at 55° no growth was obtained. Similarly, cultures one week old failed to show any resistance to heat under these conditions (55°C. for ten minutes). Kitashima and Miyajima (1) failed to infect a monkey with virulent blood which had been heated for fifteen minutes at 45°C.

Pathogenicity. The effect of these cultures on animals has been tested chiefly in monkeys and guinea-pigs since the behavior of the virus of tsutsugamushi is best understood in these animals.

Monkeys. Thus far, 3 monkeys have been inoculated with somewhat variable results. In one, a well marked fever and leucopenia appeared reaching its maximum on the tenth day; on testing this animal for immunity by inoculation with tsutsugamushi virus, a fairly definite temperature reaction appeared on the seventh day, the leucocyte curve however being atypical. Conversely, however, an animal which had reacted to the virus of tsutsugamushi showed no significant changes whatever in either temperature or leucocyte counts when injected with cultures.

In a second monkey, the injection of cultures was followed by a sharp rise in temperature on the ninth day accompanied by a leucocytosis. The behavior of the leucocytes in this individual has been very peculiar; on another occasion an extreme leucocytosis (66,000) appeared without any discernible cause. This animal was re-injected with cultures. Subsequently an

TABLE 1

Immunity test with Tsutsugamushi virus

DAYS	MONKEY IMMUNIZED WITH CULTURE		CONTROL MONKEY	
	Temperature	White count	Temperature	White count
Before injection with virus				
	102.4	19,700	102.4	26,800
	102.0	18,800	102.0	28,000
	101.8	20,300	102.2	17,000
Injected with virus				
1	102.1	66,100	104.4	38,300
2	102.5	13,200	105.2	25,000
3	104.2	12,200	104.4	20,000
4	104.0	5,300	102.6	14,300
5	103.6	6,800	102.7	17,800
6	103.4	10,100	102.8	14,000
7	102.6	10,100	103.4	10,000
8	102.0	16,800	104.2	10,400
9	102.1	12,200	106.0	9,400
10	101.3	14,900	105.5	11,200
11	102.2	21,100	105.7	10,500
12	102.8	18,500	105.3	12,200
13	103.0	25,800	105.4	4,100
14	102.1	24,400	105.4	6,400
15	101.4	24,800	104.0	8,900
16	101.6	21,000	104.4	9,000
17	102.2	24,800	102.0	8,500
18	101.8	15,000	103.0	8,000
19	102.3	18,900	101.0	7,300
20	101.8	30,600	100.7	10,700
21	101.2	19,100	101.8	7,300
22	101.2	15,100	101.4	16,900
23	100.9	28,000	100.4	17,100
24	100.3	32,100	101.6	20,300
25	101.3	26,600	101.8	22,300

injection of virulent blood was given. This immunity test was complicated by some unexplained factors but nevertheless the results indicated a substantial degree of protection. The animal injected with cultures and a normal control monkey were inoculated with virulent blood and splenic tissue from guinea-pigs. Shortly after injection, the temperature of both animals rose suddenly and returned to normal several days before the end of the incubation period for the tsutsugamushi virus. Then the control animal reacted sharply but the one previously injected with cultures showed no rise in temperature and no drop in the leucocyte count. The data are given in table 1.

In a third monkey one injection of cultures produced no definite reaction; this animal is now being re-injected with cultures in preparation ultimately for an immunity test.

It is unfortunate that a species of greater susceptibility was not available for these tests. It should be noted, however, that even the Japanese monkeys frequently fail to become infected on the first inoculation with virulent blood direct from a patient a result which is analogous to the failures occurring in rhesus monkeys on injection with blood from cases of typhus fever.

Guinea-pigs. On subcutaneous injection in guinea-pigs this organism is capable of producing hemorrhages which are very commonly diffuse, but occasionally punctate. In one instance a series of hemorrhagic spots developed along the course of one of the abdominal nerves, corresponding exactly to the picture seen in one of the pigs injected with the virus. After continuous cultivation the strains tend to lose their ability to produce these lesions; on several occasions the virulence has been restored by cultivation on fresh guinea-pig blood.

The lymph glands draining the site of inoculation are usually large and occasionally show minute hemorrhagic areas.

The spleen is sometimes enlarged and friable though this change is by no means constant.

The subcutaneous injection of the cultures produces no temperature reaction in guinea-pigs and the animals do not appear sick. Massive injections were made subcutaneously in a group of 6 pigs using for each animal the 48° growth from one-half of an ordinary Petri plate of Martin's chocolate. These injections were given daily on three successive days. Four of these animals died on the day following the last injection; a fifth died on the second and the remaining one on the third day after the last injection. A fatal result after repeated massive injections is not of itself significant. It is interesting to note however that all of these animals show a very extensive subcutaneous hemorrhagic exudate.

DISCUSSION

Clinical features of the Rickettsia diseases. In view of certain striking common characteristics, there are at present four well recognized diseases which we may place in one clinical group, namely, spotted fever of the Rocky Mountains, typhus fever, tsutsugamushi disease and trench fever. Some members of this group present a very similar clinical picture, yet the differentiation into distinct entities was made originally on clinical grounds and subsequently confirmed by laboratory investigations.

In these four infections, the virus is transmitted by a bloodsucking arthropod. The disease in man is characterized by fever and by the development of an exanthem and more or less disturbance of the nervous system. Immunologically, one attack confers well marked protection against re-infection. This protection is suggestive of sterilizing immunity (spotted and typhus fevers) though the possibility of a protracted low grade infection preventing reinfection must also be kept in mind (trench fever). The mortality is decidedly high in this group, except in trench fever which often runs a very long but nonlethal course.

It is instructive to consider the etiologic agents of this group of diseases. Ricketts (7) discovered the causative organism of spotted fever; his work was confirmed and extended by Wolbach (8) the species designation *rickettsi* being given to the organism. In typhus fever, some doubt has been expressed from time to time concerning the parasitic nature of the bodies known as

Rickettsia prowazeki. Wolbach, Pinkerton and Schlesinger (9) have recently demonstrated in tissue culture, the multiplication of the Rickettsia-like bodies of typhus fever, and the reproduction of the typical disease in guinea-pigs with the cultures. In trench fever the evidence implicating a Rickettsia type of microorganism is extremely suggestive but is as yet incomplete. The etiology of tsutsugamushi disease remains for consideration. It seems best to consider the microörganism, described in this paper, first of all in regard to its affinities with the Rickettsia group of parasites and secondly its relationship to tsutsugamushi disease.

General characteristics of Rickettsia. It is possible to describe many of the important features of some of the species of Rickettsia but our knowledge is still inadequate for an accurate definition or delimitation of the group. Many non-pathogenic forms which have been reported are more or less loosely included in this group. In general it may be said that the Rickettsia are Gram-negative, pleochromic and extremely pleomorphic organisms, many forms being characteristically bi-polar. They appear for the most part as rods or as cocco-bacillary bodies which vary markedly in size. Frequently the individual organisms are arranged in groups suggestive of the diphtheroids. In the arthropod host or in culture, long wavy chains are commonly found in some species. The cultivation of the pathogenic forms is extremely difficult. As already mentioned, the disease-producing forms with which we are acquainted produce regularly a skin rash in man, one attack affording marked protection against re-infection.

It seems to me that the organism cultivated from this strain of tsutsugamushi virus exhibits many of the essential characteristics of the bacteria. Nevertheless it corresponds closely to the typical Rickettsia of typhus fever as described and pictured very completely by Wolbach and Todd (4), by Arkwright and Bacot (10), and others. Some doubt arises as to whether the Rickettsia themselves should not be classed with the bacteria; in certain respects, however, they merit at least the dignity of a separate grouping from the ordinary bacilli. In any case, in view of the confusion concerning bacteriological nomenclature the designation of Rickettsia may well be retained for the present. Until our knowledge is more complete it seems necessary to defer the final decision as to whether the genus Rickettsia should be included in the bacteria or placed in some other classification.

The classification of the organism under consideration is of little consequence except as a possible guide in the question of its relationship to tsutsugamushi. I have no hesitation in accepting it as a hitherto unrecognized pathogenic species. On injection into guinea-pigs, lesions develop which correspond accurately with the action of the virus. The lesions produced in guinea-pigs together with the suggestive results obtained in rhesus monkeys convinces me, beyond a reasonable doubt, that this organism is the active agent of the strain of tsutsugamushi virus under investigation. There remains always for careful consideration the possibility that the original virus itself after prolonged propagation in animals, may have been supplanted or contaminated by a secondary invader. In this case, such a possibility seems to me to be remote. The Japanese investigators thoroughly familiar with the behavior of tsutsugamushi in animals had observed this strain for a period of two years. At present this virus conforms satisfactorily to the known characteristics of the disease in animals. In seems to me improbable that the cultures which have been obtained could be a contaminating microörganism which is capable of imitating the original virus in its effect on guinea-pigs and, within limits, in its effect on monkeys.

The work on this culture and on the virus in animals is obviously unfinished. However no amount of detailed work on animals alone would be final without a study of the patients themselves and also of the insect vector.

Cowdry (11) has reported negative findings in the examination of *Trombicula akamushi* for Rickettsia. A single adult individual was studied; no information is given concerning possible exposure to tsutsugamushi disease.

The characteristics of this microörganism under consideration and its effect on animals suggest to me very strongly indeed that it is the etiologic agent of the disease. The exact criteria for defining a given microörganism sometimes require years of work. For the time being it is felt that any workers encountering the microörganism described in this paper will have little difficulty in recognizing it, particular emphasis being placed on the morphology, the staining reaction, and the effects produced in guinea pigs and monkeys. For convenience sake it seems desirable to propose some designation for it. Its close affinities with the known characteristics of the Rickettsia appear to me to require placing it provisionally in that group. In the specific designation it is desired to indicate the association with Japan, its endemic home, where many Japanese workers have made brilliant contributions to our knowledge of this disease. Accordingly the name *Rickettsia nipponica* is suggested.

SUMMARY

1. A pathogenic microörganism was obtained in pure culture from animals infected with the virus of tsutsugamushi disease.
2. In morphology and staining reactions, this microörganism resembles closely the Rickettsia.
3. On inoculation of these cultures into guinea pigs, hemorrhagic lesions developed which were indistinguishable from the hemorrhages following the injection of the virus of tsutsugamushi disease. The cultures were pathogenic, to some extent for rhesus monkeys, the injected animals showing some evidence of active immunity against the virus.
4. There has, as yet, been no opportunity to control these observations by the study of tsutsugamushi disease in man or by the examination of infective mites.

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To Dr. Otto Schöbl of the Bureau of Science in Manila, I am indebted for invaluable assistance in Japan.

SUMARIO

1. Se obtuvo un microorganismo patógeno, en cultivo puro, de animales infectados con el virus de la enfermedad de tsutsugamushi.

2. En morfología y teñido este microorganismo se parece mucho al de la Rickettsia.

3. La inoculación de estos cultivos en cobayos produjo lesiones hemorrágicas que no se podían distinguir de las hemorragias que produce la inyección del virus de la enfermedad de tsutsugamushi. Los cultivos tenían patogenicidad para los monos *rhesus* los cuales presentaban evidencia de inmunidad activa en contra del virus.

4. Aún no se ha presentado la oportunidad de poder testificar estas observaciones mediante el estudio de la enfermedad en el hombre o por el exámen de los ácaros infecciosos.

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PLATE 1

MICROÖRGANISMS CULTIVATED FROM ANIMALS INFECTED WITH TSUTSUGAMUSHI VIRUS

The preparations were stained with a well aged Loffler's methylene blue. The magnification is 1500 diameters. The drawings were made by Miss Etta Piotti with the accuracy characteristic of her work.

Fig. 1. Typical forms from a culture growing freely on Martin's chocolate agar.

Fig. 2. A culture from meat infusion agar showing pleomorphism.

Fig. 3. Minute forms from Hiss-serum water.

Fig. 4. Faintly staining forms from a fifteen-hour transplant on glucose agar. These are for the present interpreted as young forms of this organism.

A RICKETTSIA-LIKE MICROÖRGANISM
ANDREW WATSON SELLARDS

PLATE 1

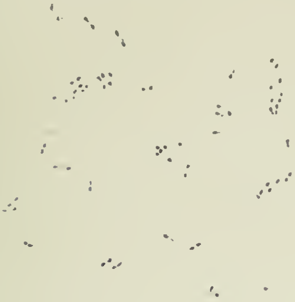

Fig. 1

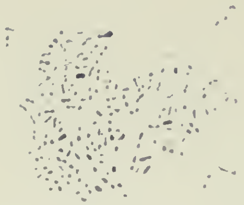

Fig. 3

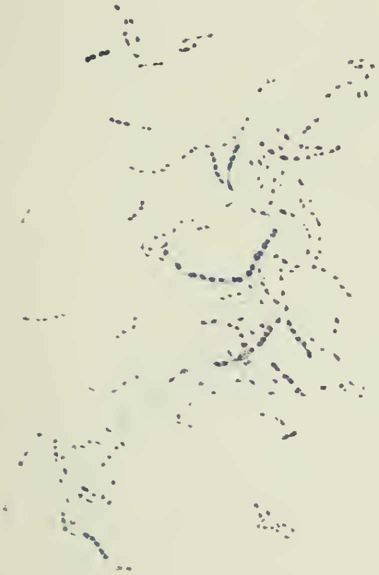

Fig. 2

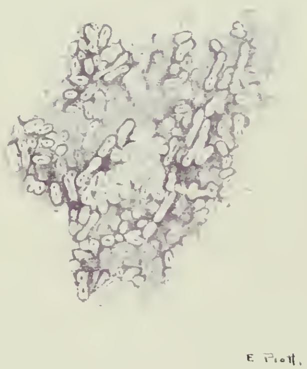

Fig. 4

BOOK REVIEW

Cerebrospinal Fluid in Health and in Disease. By ABRAHAM LEVINSON, B.S., M.D., Associate in Pediatrics, Northwestern University Medical School; Attending Physician, Department of Contagious Diseases, Cook County Hospital, Chicago; Attending Pediatrician, Sarah Morris Hospital for Children of the Michael Reese Hospital, Chicago; Attending Pediatrician, Mount Sinai Hospital, Chicago. With a fore-word by Ludwig Hektoen, M.D. With sixty-five illustrations, including five colored plates. Second edition, thoroughly revised. St. Louis, C. V. Mosby Co. 1923. 267 pages. Price $5.00.

This work furnishes an excellent reference book for the laboratory worker, and has a value as a text book especially in post graduate courses in neuropathology. A short history in the first chapter, with its bibliography, is an excellent résumé of the historical high lights in investigations of the cerebrospinal fluid. The second chapter abstracts the recent theories of physiology and affords a valuable anatomical working knowledge for research workers along these lines. Chapter III, with its illustrations of apparatus and instruments, is one of the best descriptions of the mechanics of spinal and ventricular puncture in the literature.

The physico-chemical handling in Chapters IV and V is made most complete by its tables and charts which give it a high reference value to the laboratory worker. The application of the knowledge in these chapters, make this book most useful in helping the laboratory investigator of spinal fluids to steer clear from the errors which creep into this procedure. The illustration and explanation of the gold curve is a most lucid one and lends itself admirably to teaching purposes. The chapter on treatment is especially valuable since in most of the smaller hospitals the clinician usually turns to the pathological service for help in the cerebrospinal cases. The bibliography which appears at the end of each chapter is of value to those investigating along these lines.

JAMES F. COUPAL.

Army Medical Museum.

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